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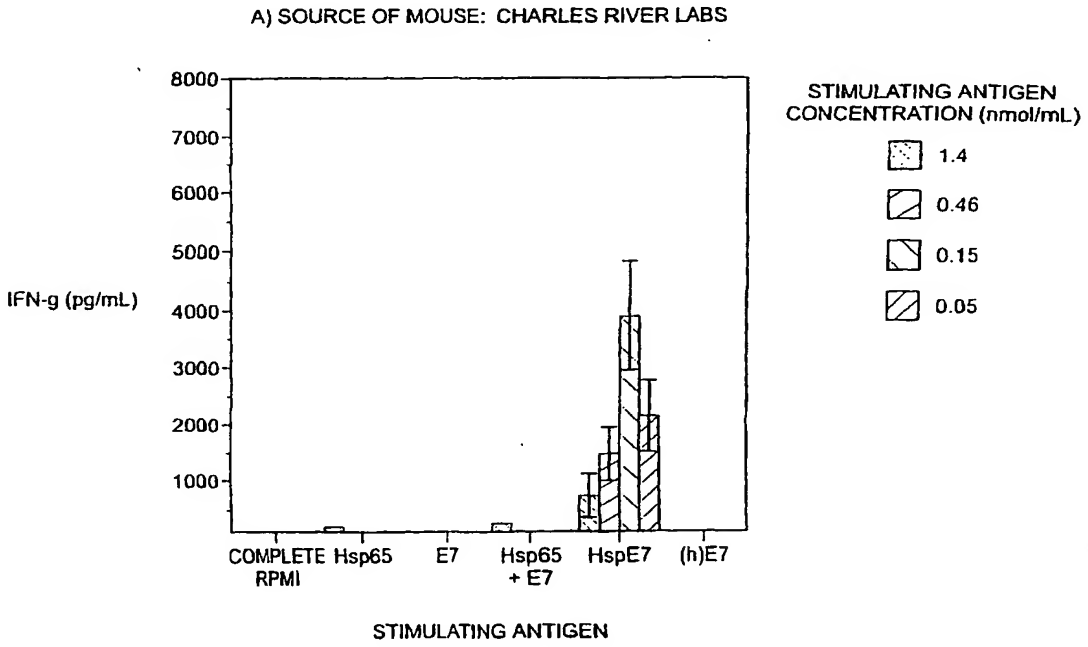
(54) Title: INDUCTION OF A TH1-LIKE RESPONSE IN VITRO



(57) Abstract: The invention provides compositions and methods for stimulating a Th1-like response *in vitro*. Compositions include fusion proteins and conjugates that contain at least a portion of a heat shock protein. A Th1-like response can be elicited by contacting *in vitro* a cell sample containing naive lymphocytes with a fusion protein or conjugate of the invention. The Th1-like response can be detected by measuring IFN-gamma produced by the cell sample.

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INDUCTION OF A TH1-LIKE RESPONSE IN VITRO

Cross Reference to Related Applications

This application claims priority from U.S. Provisional Application No. 60/143,757, filed July 8, 1999. The content of this application is incorporated herein by reference in its entirety.

Field of the Invention

The invention relates to fusion proteins and methods of stimulating a Th1-like response *in vitro*.

Background

T lymphocytes can generally be divided into two classes based upon expression of the CD4 and CD8 antigens. The immune response mediated by CD4+ T cells is restricted by class II major histocompatibility complex (MHC) molecules. CD4+ T cells, also known as helper T lymphocytes, carry out their helper functions via the secretion of lymphokines. The immune response mediated by CD8+ T cells is restricted by class I MHC molecules. CD8+ T cells, also known as cytolytic T lymphocytes (CTLs), carry out cell mediated cytotoxicity and also secrete some lymphokines upon activation.

CD4+ T cells can be further divided into Th1 and Th2 subsets. Th1 cells participate in cell mediated immunity by producing lymphokines, such as interferon (IFN)-gamma and tumor necrosis factor (TNF)-beta, that activate cell mediated immunity. Th2 cells provide help for humoral immunity by secreting lymphokines that stimulate B cells, such as IL-4 and IL-5. Antigenic stimuli that activate either the Th1 or Th2 pathway can inhibit the development of the other. For example, IFN-gamma produced by a stimulated Th1 cell can inhibit the formation of Th2 cells, and IL-4 produced by a stimulated Th2 cell can inhibit the formation of Th1 cells.

Certain disease conditions, such as cancer, allergy, and parasitic infections, are characterized by a predominantly Th2 response. Under certain circumstances, the induction of the Th1 response, typified by the production of IFN-gamma, may ameliorate these conditions.

Summary of the Invention

The invention is based on the discovery that a cell sample containing naive lymphocytes can be stimulated *in vitro* to exhibit a Th1-like response.

Accordingly, the invention features a method of determining whether a fusion protein stimulates a Th1-like response by: (a) providing a cell sample containing naive lymphocytes *in vitro*; (b) providing a fusion protein containing (i) a heat shock protein (Hsp) or a fragment thereof at least eight amino acid residues in length, fused to (ii) a heterologous polypeptide at least eight amino acid residues in length; (c) contacting the cell sample with the fusion protein; and (d) determining whether the fusion protein stimulates a Th1-like response in the cell sample.

"Naive lymphocytes" are lymphocytes that have not been exposed to the fusion protein (*in vivo* or *in vitro*) prior to their use in a method the invention. An "Hsp" is a polypeptide consisting of a sequence that is at least 40% identical to that of a protein whose expression is induced or enhanced in a cell exposed to stress, e.g., heat shock. A "fusion protein" is a non-naturally occurring polypeptide containing amino acid sequences derived from at least two different proteins.

The Hsp used in the method can be selected from the group consisting of Hsp65, Hsp40, Hsp10, Hsp60, and Hsp71. Additionally, the fusion protein can contain the full amino acid sequence of any of Hsp65, Hsp40, Hsp10, Hsp60, or Hsp71. In some embodiments, the fusion protein contains a fragment of an Hsp, e.g., amino acids 1-200 of Hsp65 of *Mycobacterium bovis*.

The heterologous polypeptide can contain a sequence identical to at least eight consecutive amino acids of (i) a protein of a human pathogen, e.g., a virus, or (ii) a tumor associated antigen. Examples of viruses include human papilloma virus (HPV), herpes simplex virus (HSV), hepatitis B virus (HBV), hepatitis C virus (HCV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), influenza virus, measles virus, and human immunodeficiency virus (HIV). The heterologous polypeptide can contain an HPV E6 antigen, e.g., HPV16 E6, an HPV E7 antigen, e.g., HPV16 E7, or a fragment of any of these antigens that is at least eight amino acid residues in length.

In one example, the fusion protein contains *Mycobacterium bovis* BCG Hsp65 and HPV 16 E7.

The cell sample used in the methods of the invention can contain cells derived from a spleen, lymph node, peripheral blood, bone marrow, thymus, lung, respiratory

tract, or anogenital mucosa. In preferred embodiments, the cells are splenocytes or lymph node cells.

The stimulation of a Th1-like response can be determined by detecting the presence of a lymphokine produced by the cell sample, e.g. IFN-gamma or TNF-beta.

In one embodiment, the method also includes the steps of: (e) providing a second cell sample containing naive lymphocytes; (f) contacting the second cell sample with a second fusion protein; and (g) determining whether the second fusion protein stimulates a Th1-like response in the second cell sample. In this example, the first fusion protein contains the sequence of a full-length, naturally occurring Hsp, and the second fusion protein contains at least eight amino acids but less than all of the sequence of a naturally occurring Hsp.

In another aspect, the invention features a method of screening a compound by: (a) providing a cell sample containing naive lymphocytes *in vitro*; (b) providing a fusion protein containing (i) a Hsp or a fragment thereof at least eight amino acid residues in length, fused to (ii) a heterologous polypeptide at least eight amino acid residues in length; (c) contacting the cell sample with the compound and the fusion protein; and (d) determining whether the cell sample exhibits a Th1-like response following the contacting step. In this method, a decrease in the Th1-like response in the presence of the compound compared to in the absence of the compound indicates that the compound inhibits a Th1-like response by the cell sample.

The invention also includes a method of screening a compound by: (a) providing a cell sample containing naive lymphocytes *in vitro*; (b) providing a fusion protein containing (i) a Hsp or a fragment thereof at least eight amino acid residues in length, fused to (ii) a heterologous polypeptide at least eight amino acid residues in length; (c) contacting the cell sample with the compound and the fusion protein; and (d) determining whether the cell sample exhibits a Th1-like response following the contacting step. In this method, an increase in the Th1-like response in the presence of the compound compared to in the absence of the compound indicates that the compound promotes a Th1-like response by the cell sample.

In another aspect, the invention features a method of determining whether a hybrid compound stimulates a Th1-like response by: (a) providing a cell sample containing naive lymphocytes *in vitro*; (b) providing a hybrid compound that is non-naturally occurring and contains (i) a non-peptide compound having a molecular weight

of less than 1,500, covalently linked to (ii) a polypeptide of at least eight amino acids in length, wherein the hybrid compound is made by covalently linking the non-peptide compound to the polypeptide; (c) contacting the cell sample with the hybrid compound; and (d) determining whether the hybrid compound stimulates a Th1-like response in the cell sample. In one embodiment, the non-peptide compound has a molecular weight of at least 100.

In another aspect, the invention features a method of determining whether a hybrid compound stimulates a Th1-like response by: (a) producing a hybrid compound by covalently linking a non-peptide compound to a polypeptide of at least eight amino acids in length; (b) providing a cell sample containing naive lymphocytes *in vitro*; (c) contacting the cell sample with the hybrid compound; and (d) determining whether the hybrid compound stimulates a Th1-like response in the cell sample. In one embodiment, the non-peptide compound has a molecular weight between 100 and 1,500.

In another aspect, the invention features a method of determining whether a fusion protein stimulates a Th1-like response by: (a) providing a cell sample containing naive lymphocytes *in vitro*; (b) providing a fusion protein comprising (i) a first polypeptide at least eight amino acids in length, fused to (ii) a second polypeptide at least eight amino acids in length; (c) contacting the cell sample with the fusion protein; and (d) detecting a Th1-like response exhibited by the cell sample following the contacting step. In one embodiment, the detected Th1-like response is greater than a Th1-like response exhibited by a second cell sample containing naive lymphocytes when the second cell sample is contacted with either the first polypeptide, the second polypeptide, or a mixture of the first polypeptide and the second polypeptide. In one example, the detected Th1-like response is at least two times greater than the Th1-like response exhibited by the second cell sample. In another example, the detected Th1-like response is at least five times greater than the Th1-like response exhibited by the second cell sample.

In another aspect, the invention provides a fusion protein containing (i) a Hsp10 protein or a fragment thereof at least eight amino acid residues in length, and (ii) a heterologous polypeptide at least eight amino acids in length. The Hsp10 protein of the fusion protein can be a mycobacterial protein, e.g., *Mycobacterium tuberculosis* Hsp10 protein. The heterologous polypeptide can contain a sequence identical to at least eight

consecutive amino acids of a protein of a human virus, e.g., HPV. In one example, the heterologous polypeptide contains HPV16 E7.

In another aspect, the invention provides a fusion protein containing (i) a Hsp40 protein or a fragment thereof at least eight amino acid residues in length, and (ii) a heterologous polypeptide at least eight amino acids in length. The Hsp40 protein of the fusion protein can be a mycobacterial protein, e.g., *Mycobacterium tuberculosis* Hsp40 protein. The heterologous polypeptide can contain a sequence identical to at least eight consecutive amino acids of a protein of a human virus, e.g., HPV. In one example, the heterologous polypeptide contains HPV16 E7.

In another aspect, the invention provides a fusion protein containing (i) a Hsp71 protein or a fragment thereof at least eight amino acid residues in length, and (ii) a heterologous polypeptide at least eight amino acids in length. The Hsp71 protein of the fusion protein can be a mycobacterial protein, e.g., *Mycobacterium tuberculosis* Hsp71 protein. The heterologous polypeptide can contain a sequence identical to at least eight consecutive amino acids of a protein of a human virus, e.g., HPV. In one example, the heterologous polypeptide contains HPV16 E7.

In another aspect, the invention features a method of determining whether a compound stimulates a Th1-like response by: (a) providing a cell sample containing naive lymphocytes *in vitro*; (b) providing a compound; (c) contacting the cell sample with the compound; and (d) detecting a Th1-like response exhibited by the cell sample following the contacting step.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present application, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

Brief Description of the Drawings

Figures 1A-1B show the sequence of plasmid pET65 coding for expression of Hsp65.

Figure 2 shows the sequence of plasmid pET/E7 (NH) coding for expression of E7.

Figure 3 shows the sequence of plasmid pET/H/E7 coding for expression of (h)E7.

Figures 4A-4B show the sequence of plasmid pET65C/E7-1N coding for expression of HspE7.

Figures 5A-5B show the sequence of plasmid pETMT40E7 coding for expression of MT40-E7.

Figure 6 shows the sequence of plasmid pET/OVA coding for expression of ovalbumin (OVA).

Figures 7A-7C show the sequence of plasmid pET65H/OVA coding for expression of HspOVA.

Figure 8 shows the sequence of plasmid pGEX/K coding for expression of GST.

Figure 9 shows the sequence of plasmid pGEX/K/E7 coding for expression of GST-E7.

Figures 10A-10B show the sequence of plasmid pET/E7/5'65 coding for expression of E7-L-BCG65.

Figure 11 shows the sequence of plasmid pET65F1/E7 coding for expression of BCG65(F1)-E7.

Figure 12 shows the sequence of plasmid pETESE7 coding for expression of TB10-E7.

Figures 13A-13B show the sequence of plasmid pET/E7/71 coding for expression of E7-TB71.

Figures 14A-14B show the sequence of plasmid pET/E7/71' coding for expression of a fusion protein.

Figures 15A-15B show the sequence of plasmid pET/SP65c-E7 coding for expression of SP65(2)-E7.

Figures 16A-16B show the sequence of plasmid pETAF60E7 coding for expression of AF60-E7.

Figures 17A-17B show enhanced IFN-gamma release by splenocytes from C57BL/6 mice obtained from the Charles River Laboratory (Fig. 17A) and the Jackson Laboratory (Fig. 17B) upon exposure to HspE7.

Figures 18A-18C show enhanced IFN-gamma release by splenocytes from Balb/c (Fig. 18A), C57BL/6 (Fig. 18B), and C3HeB/FeJ (Fig. 18C) mice upon exposure to HspE7.

Figure 19 shows enhanced IFN-gamma release by splenocytes upon exposure to fusion proteins containing an antigen and a stress protein but not upon exposure to a fusion protein containing an antigen and a protein other than a stress protein.

Figures 20A-20B show enhanced IFN-gamma release by splenocytes upon exposure to fusion proteins containing stress proteins of different types, stress proteins from different organisms, or a fragment of a stress protein.

Figure 21 shows enhanced IFN-gamma release by lymph node cells and splenocytes upon exposure to fusion proteins containing an antigen and a stress protein.

Figures 22A-22B show a time course of tumor incidence (Fig. 22A) and tumor volume (Fig. 22B) in mice injected with TC-1 tumor cells followed by an injection with either saline, HspE7, SP65(2)-E7, or AF60-E7.

Figures 23A-23B show a time course of tumor incidence (Fig. 23A) and tumor volume (Fig. 23B) in mice injected with TC-1 tumor cells followed by an injection with either saline, HspE7, MT40-E7, E7-MT71, or TB10-E7.

Detailed Description

The invention relates to methods of stimulating *in vitro* a Th1-like response in a cell sample containing naive lymphocytes. These methods are useful for assessing the ability of a protein, e.g., a fusion protein containing an Hsp linked to a heterologous polypeptide, to function as a stimulator of a Th1-like response. Additionally, the method can be used to identify compounds that can regulate a Th1-like response. Various materials and procedures suitable for use in the methods of the invention are discussed below.

The terms stress protein and heat shock protein (Hsp) are used synonymously herein. An Hsp is a polypeptide consisting of a sequence that is at least 40% identical to that of a protein whose expression is induced or enhanced in a cell exposed to stress. Turning to stress proteins generally, cells respond to a stressor (typically heat shock

treatment) by increasing the expression of a group of genes commonly referred to as stress, or heat shock, genes. Heat shock treatment involves exposure of cells or organisms to temperatures that are one to several degrees Celsius above the temperature to which the cells are adapted. In coordination with the induction of such genes, the levels of corresponding stress proteins increase in stressed cells. As used herein, a "stress protein," also known as a "heat shock protein" or "Hsp," is a protein that is encoded by a stress gene, and is therefore typically produced in significantly greater amounts upon the contact or exposure of the stressor to the organism. A "stress gene," also known as "heat shock gene" is used herein as a gene that is activated or otherwise detectably upregulated due to the contact or exposure of an organism (containing the gene) to a stressor, such as heat shock, hypoxia, glucose deprivation, heavy metal salts, inhibitors of energy metabolism and electron transport, and protein denaturants, or to certain benzoquinone ansamycins. Nover, L., *Heat Shock Response*, CRC Press, Inc., Boca Raton, FL (1991). "Stress gene" also includes homologous genes within known stress gene families, such as certain genes within the Hsp70 and Hsp90 stress gene families, even though such homologous genes are not themselves induced by a stressor. Each of the terms stress gene and stress protein as used in the present specification may be inclusive of the other, unless the context indicates otherwise.

An antigen can be any compound, peptide or protein to which an immune response is desired. Antigens of particular interest are tumor-associated antigens, allergens of any origin, and proteins from viruses, mycoplasma, bacteria, fungi, protozoa and other parasites.

Fusion Proteins

The invention provides Hsp fusion proteins. As used herein, a "fusion protein" is a non-naturally occurring polypeptide containing at least two amino acid sequences which generally are from two different proteins. The amino acid sequence of the full length fusion protein is not identical to the amino acid sequence of a naturally occurring protein or a fragment thereof. An Hsp fusion protein contains an Hsp or a fragment thereof at least eight amino acids in length linked to a heterologous polypeptide. An "Hsp polypeptide" refers to a polypeptide consisting of a sequence that is at least 40% identical to that of a protein whose expression is induced or enhanced in a cell exposed to stress, e.g., heat shock. A "heterologous polypeptide" refers to a polypeptide that is

fused to the Hsp protein or fragment thereof. The heterologous polypeptide is preferably at least eight amino acids in length. In some embodiments, the heterologous polypeptide is at least 10, 20, 50, 100, 150, 180, 200, or 300 amino acids in length. The heterologous polypeptide generally is not part or all of a naturally occurring Hsp. However, the fusion protein can also be a fusion between a first Hsp and a second, different, Hsp, or between all or portion of an Hsp fused to all or a portion of the same Hsp (as long as the resultant fusion is not identical to a naturally occurring protein). The Hsp polypeptide can be attached to the N-terminus or C-terminus of the heterologous polypeptide. Preferably the fusion protein is a purified protein.

The preferred Hsp fusion protein has one Hsp polypeptide linked to one heterologous polypeptide, but other conformations are within the invention. In one embodiment, the fusion protein comprises at least two copies of the heterologous polypeptide, e.g., HPV16 E7. In another embodiment, the fusion protein contains at least two copies of the Hsp polypeptide, e.g., Hsp65. Additionally, the fusion protein can contain at least two different heterologous polypeptides, e.g., two or more fragments of a single antigenic protein representing different epitopes or fragments of two or more different antigenic proteins derived from the same or different tumors or pathogens, and/or at least two different Hsp polypeptides.

The Hsp and heterologous polypeptide can be directly fused without a linker sequence. In preferred embodiments, the C-terminus of the Hsp can be directly fused to the N-terminus of the heterologous polypeptide or the C-terminus of the heterologous polypeptide can be directly fused to the N-terminus of the Hsp.

Alternatively, Hsp and heterologous polypeptides can be linked to each other via a peptide linker sequence. Preferred linker sequences (1) should adopt a flexible extended conformation, (2) should not exhibit a propensity for developing an ordered secondary structure which could interact with the functional Hsp and heterologous polypeptide domains, and (3) should have minimal hydrophobic or charged character, which could promote interaction with the functional protein domains. Typical surface amino acids in flexible protein regions include Gly, Asn and Ser. Permutations of amino acid sequences containing Gly, Asn and Ser would be expected to satisfy the above criteria for a linker sequence. Other neutral or near-neutral amino acids, such as Thr and Ala, can also be used in the linker sequence. Any other amino acid can also be used in the linker. A linker sequence length of fewer than 20 amino acids can be used

to provide a suitable separation of functional protein domains, although longer linker sequences may also be used.

The Hsp fusion protein may be further fused to another amino acid sequence that facilitates the purification of the fusion protein. One useful fusion protein is a GST fusion protein in which the Hsp-heterologous polypeptide sequences are fused to the C-terminus or N-terminus of the GST sequence. Another useful fusion protein is a poly-histidine (His) fusion protein in which the Hsp-heterologous polypeptide sequences are fused to either the C-terminus or N-terminus of the poly-histidine sequence, e.g. His x 6. In another embodiment, the fusion protein contains the chitin-binding region of intein, thereby permitting the purification of the fusion protein by chitin beads (Hoang et al. (1999) *Gene* 1999 237:361-71). In another embodiment, the fusion protein contains a signal sequence from another protein. In certain host cells (e.g., mammalian host cells), expression and/or secretion of the Hsp fusion protein can be increased through use of a heterologous signal sequence. For example, the gp67 secretory sequence of the baculovirus envelope protein can be used as a heterologous signal sequence (Current Protocols in Molecular Biology, Ausubel et al., eds., John Wiley & Sons, 1992). Other examples of eukaryotic signal sequences include the secretory sequences of melittin and human placental alkaline phosphatase (Stratagene; La Jolla, California). Prokaryotic signal sequences useful for increasing secretion by a prokaryotic host cell include the phoA secretory signal (Molecular Cloning, Sambrook et al., second edition, Cold Spring Harbor Laboratory Press, 1989) and the protein A secretory signal (Pharmacia Biotech; Piscataway, New Jersey).

Fusion proteins of the invention, e.g., a fusion protein of Hsp65 and HPV16 E7, can be produced by standard recombinant techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together, in any order, in-frame in accordance with conventional techniques. Such techniques can include employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. Correct linkage of the two nucleic acids requires that the product of the linkage encode a chimeric protein consisting of a Hsp moiety and a heterologous polypeptide moiety. In another embodiment, the fusion gene can be synthesized by conventional techniques, including automated DNA synthesizers. Alternatively, PCR amplification of gene

fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments, which are subsequently annealed and reamplified to generate a chimeric gene sequence (see, e.g., Current Protocols in Molecular Biology, Ausubel et al. eds., John Wiley & Sons: 1992).

Expression vectors encoding fusion proteins containing a heterologous polypeptide and either an Hsp or a protein other than an Hsp can be prepared by the above procedures. Examples of Hsp fusion proteins can be found in international patent application WO 99/07860, incorporated herein by reference, that describes vector construction, expression and purification of *Mycobacterium bovis* BCG Hsp65 - HPV16 E7 (HspE7) fusion protein as well as of HPV16 E7 (E7), histidine tagged HPV16 E7 (hE7), and *M. bovis* BCG Hsp65 (Hsp65). Additional examples of nucleic acids encoding an Hsp optionally linked to a heterologous polypeptide, e.g., an HPV antigen, are described in WO 89/12455, WO 94/29459, WO 98/23735, and references cited therein, the contents of which are herein incorporated by reference.

A variety of heat shock proteins have been isolated, cloned, and characterized from a diverse array of organisms (Mizzen, Biotherapy 10:173-189, 1998). Any Hsp or fragment thereof may be suitable for use in the fusion polypeptides and conjugates of the invention. For example, Hsp70, Hsp60, Hsp20-30, and Hsp10 are among the major determinants recognized by host immune responses to infection by *Mycobacterium tuberculosis* and *Mycobacterium leprae*. In addition, Hsp65 of Bacille Calmette Guerin (BCG), a strain of *Mycobacterium bovis*, was found to be an effective stimulatory agent, as described in the examples below.

Families of stress genes and proteins for use in the present invention are well known in the art and include, for example, Hsp100-200, Hsp100, Hsp90, Lon, Hsp70, Hsp60, TF55, Hsp40, FKBPs, cyclophilins, Hsp20-30, ClpP, GrpE, Hsp10, ubiquitin, calnexin, and protein disulfide isomerases. See, e.g., Macario, Cold Spring Harbor Laboratory Res. 25:59-70, 1995; Parsell et al., Rev. Genet. 27:437-496, 1993; and U.S. Patent No. 5,232,833. Preferred Hsps include Hsp65, Hsp40, Hsp10, Hsp60, and Hsp71.

The Hsp portion of the fusion protein can include either a full length Hsp or a fragment of an Hsp at least eight amino acids in length. In some embodiments, the Hsp fragment is greater than 10 amino acids in length, and preferably is at least 20, 50, 100, 150, 180, 200, or 300 amino acids in length. In one embodiment, the Hsp portion of the

fusion protein consists of amino acids 1-200 of Hsp65 of *Mycobacterium bovis*. Other portions of Hsp65 and other Hsps can be used in a fusion protein to elicit a Th1-like response *in vitro*. Other preferred Hsps include Hsp40 of *M. tuberculosis*, Hsp10 of *M. tuberculosis*, Hsp65 of *Streptococcus pneumoniae*, and Hsp60 of *Aspergillus fumigatus*.

Heterologous polypeptides can contain any amino acid sequence useful for stimulating an immune response, *in vitro* and/or *in vivo*. Preferably, the heterologous polypeptide contains an MHC-binding epitope, e.g., an MHC class I or MHC class II binding epitope. The heterologous polypeptide can contain sequences found in a protein produced by a human pathogen, e.g., viruses, bacteria, mycoplasma, fungi, protozoa, and other parasites, or sequences found in the protein of a tumor associated antigen (TAA). Examples of viruses include human papilloma virus (HPV), herpes simplex virus (HSV), hepatitis B virus (HBV), hepatitis C virus (HCV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), influenza virus, measles virus, and human immunodeficiency virus (HIV). Examples of tumor associated antigens include MAGE1, MAGE2, MAGE3, BAGE, GAGE, PRAME, SSX-2, Tyrosinase, MART-1, NY-ESO-1, gp100, TRP-1, TRP-2, A2 melanotope, BCR/ABL, Proeinase-3/Myeloblastin, HER2/neu, CEA, P1A, HK2, PAPA, PSA, PSCA, PSMA, pg75, MUM-1, MUC-1, E6, E7, GnT-V, Beta-catenin, CDK4 and P15.

HPV antigens from any strain of HPV are suitable for use in the fusion polypeptide. HPV expresses six or seven non-structural and two structural proteins. Viral capsid proteins L1 and L2 are the late structural proteins. L1 is the major capsid protein, the amino acid sequence of which is highly conserved among different HPV types. There are seven early non-structural proteins. Proteins E1, E2, and E4 play an important role in virus replication. Protein E4 also plays a role in virus maturation. The role of E5 is less well known. Proteins E6 and E7 are oncoproteins critical for viral replication, as well as for host cell immortalization and transformation. Fusion proteins of the invention can contain either the entire sequence of an HPV protein or a fragment thereof, e.g., a fragment of at least 8 amino acids. In one embodiment, the HPV antigenic sequence is derived from a "high risk" HPV, such as HPV16 or HPV18 E7 protein. The HPV antigenic sequence can include an MHC-binding epitope, e.g., an MHC class I and/or an MHC class II binding epitope.

In addition to Hsp fusion proteins, other fusion proteins can be used in the *in vitro* assay described herein. These non-Hsp fusion proteins contain a first polypeptide at least eight amino acids in length, fused to a second polypeptide at least eight amino acids in length, wherein the first and second polypeptides are derived from different proteins (preferably naturally occurring proteins). The fusion protein itself does not have the sequence of a naturally occurring protein.

In the fusion protein of the invention, neither the first nor second polypeptide is an amino acid sequence that is commonly used for protein purification or detection, e.g., GST or poly-histidine.

In order to produce the fusion protein, a nucleic acid encoding the fusion protein can be introduced into a host cell, e.g., a bacterium, a primary cell, or an immortalized cell line using an expression vector. The recombinant cells are then used to produce the fusion protein. The transfection can be transient or stable, the later sometimes accomplished by homologous recombination.

The nucleotide sequence encoding a fusion protein will usually be operably linked to one or more regulatory sequences, selected on the basis of the host cells to be used for expression. The term "regulatory sequence" refers to promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel (1990) *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA, the content of which is incorporated herein by reference. Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cells, those that direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences), and those that direct expression in a regulatable manner (*e.g.*, only in the presence of an inducing agent). It will be appreciated by those skilled in the art that the design of the expression vector may depend on such factors as the choice of the host cell to be transformed, the level of expression of fusion protein desired, and the like.

Recombinant expression vectors can be designed for expression of fusion proteins in prokaryotic or eukaryotic cells. For example, fusion proteins can be expressed in bacterial cells such as *E. coli*, insect cells (e.g., in the baculovirus expression system), yeast cells or mammalian cells. Some suitable host cells are discussed further in Goeddel (1990) *Gene Expression Technology: Methods in*

Enzymology 185, Academic Press, San Diego, CA. Examples of vectors for expression in yeast *S. cerevisiae* include pYepSec1 (Baldari *et al.* (1987) *EMBO J.* 6:229-234), pMFa (Kurjan and Herskowitz (1982) *Cell* 30:933-943), pJRY88 (Schultz *et al.* (1987) *Gene* 54:113-123), and pYES2 (Invitrogen Corporation, San Diego, CA). Baculovirus vectors available for expression of fusion proteins in cultured insect cells (*e.g.*, Sf 9 cells) include the pAc series (Smith *et al.* (1983) *Mol. Cell. Biol.* 3:2156-2165) and the pVL series (Lucklow and Summers (1989) *Virology* 170:31-39).

Examples of mammalian expression vectors include pCDM8 (Seed (1987) *Nature* 329:840) and pMT2PC (Kaufman *et al.* (1987), *EMBO J.* 6:187-195). When intended for use in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40.

In addition to the regulatory control sequences discussed above, the recombinant expression vector can contain additional nucleotide sequences. For example, the recombinant expression vector may encode a selectable marker gene to identify host cells that have incorporated the vector. Moreover, to facilitate secretion of the fusion protein from a host cell, in particular mammalian host cells, the recombinant expression vector can encode a signal sequence linked to the amino-terminus of the fusion protein, such that upon expression, the fusion protein is synthesized with the signal sequence fused to its amino terminus. This signal sequence directs the fusion protein into the secretory pathway of the cell and is then usually cleaved, allowing for release of the mature fusion protein (*i.e.*, the fusion protein without the signal sequence) from the host cell. Use of a signal sequence to facilitate secretion of proteins or peptides from mammalian host cells is known in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells *via* conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, electroporation, microinjection and viral-mediated transfection. Suitable methods for transforming or transfecting host cells can be found in Sambrook *et al.* (*Molecular Cloning: A Laboratory Manual*, 2nd Edition, Cold Spring Harbor Laboratory Press (1989)), and other laboratory manuals.

Often only a small fraction of mammalian cells integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, resistance to antibiotics) can be introduced into the host cells along with the gene encoding the fusion protein. Preferred selectable markers include those that confer resistance to drugs such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding the fusion protein or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

Alternatively, a recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

In addition to the recombinant techniques described above, a fusion protein of the invention can be formed by linking two polypeptides, e.g., a Hsp and a heterologous polypeptide, to form a conjugate. Methods of forming Hsp conjugates are described in WO 89/12455, WO 94/29459, WO 98/23735, and WO 99/07860, the contents of which are herein incorporated by reference. As used herein, an Hsp "conjugate" comprises an Hsp that has been covalently linked to a heterologous polypeptide via the action of a coupling agent. A conjugate thus comprises two separate molecules that have been coupled one to the other. The term "coupling agent," as used herein, refers to a reagent capable of coupling one polypeptide to another polypeptide, e.g., a Hsp to a heterologous polypeptide. Any bond which is capable of linking the components such that the linkage is stable under physiological conditions for the time needed for the assay (e.g., at least 12 hours, preferably at least 72 hours) is suitable. The link between two components may be direct, e.g., where a Hsp is linked directly to a heterologous polypeptide, or indirect, e.g., where a Hsp is linked to an intermediate, e.g., a backbone, and that intermediate is also linked to the heterologous polypeptide. A coupling agent should function under conditions of temperature, pH, salt, solvent system, and other reactants that substantially retain the chemical stability of the Hsp, the backbone (if present), and the heterologous polypeptide.

A coupling agent can link components, e.g., a Hsp and a heterologous polypeptide, without the addition of the coupling agent to the resulting fusion protein. Other coupling agents result in the addition of the coupling agent to the resulting fusion

protein. For example, coupling agents can be cross-linking agents that are homo- or hetero-bifunctional, and wherein one or more atomic components of the agent is retained in the composition. A coupling agent that is not a cross-linking agent can be removed entirely following the coupling reaction, so that the molecular product is composed entirely of the Hsp, the heterologous polypeptide, and a backbone moiety (if present).

Many coupling agents react with an amine and a carboxylate, to form an amide, or an alcohol and a carboxylate to form an ester. Coupling agents are known in the art, see, e.g., M. Bodansky, "Principles of Peptide Synthesis", 2nd ed., referenced herein, and T. Greene and P. Wuts, "Protective Groups in Organic Synthesis," 2nd Ed, 1991, John Wiley, NY. Coupling agents should link component moieties stably, but such that there is minimal or no denaturation or deactivation of the Hsp or the heterologous polypeptide.

The conjugates of the invention can be prepared by coupling a Hsp to a heterologous polypeptide using methods known in the art. A variety of coupling agents, including cross-linking agents, can be used for covalent conjugation. Examples of cross-linking agents include N,N'-dicyclohexylcarbodiimide (DCC; Pierce), N-succinimidyl-S-acetyl-thioacetate (SATA), N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), ortho-phenylenedimaleimide (*o*-PDM), and sulfosuccinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (sulfo-SMCC). See, e.g., Karpovsky *et al.* (1984) *J. Exp. Med.* 160:1686 and Liu *et al.* (1985) *Proc. Natl. Acad. Sci. USA 82*:8648. Other methods include those described by Paulus (1985) *Behring Ins. Mitt.* 78:118-132; Brennan *et al.* (1985) *Science* 229:81-83; and Glennie *et al.* (1987) *J. Immunol.* 139: 2367-2375. A large number of coupling agents for peptides and proteins, along with buffers, solvents, and methods of use, are described in the Pierce Chemical Co. catalog, pages T-155 –T-200, 1994 (3747 N. Meridian Rd., Rockford IL, 61105, U.S.A.; Pierce Europe B.V., P.O. Box 1512, 3260 BA Oud Beijerland, The Netherlands), which catalog is hereby incorporated by reference.

DCC is a useful coupling agent (Pierce #20320; Rockford, IL). It promotes coupling of the alcohol NHS in DMSO (Pierce #20684), forming an activated ester which can be cross-linked to polylysine. DCC (N,N'-dicyclohexylcarbodiimide) is a carboxy-reactive cross-linker commonly used as a coupling agent in peptide synthesis,

and has a molecular weight of 206.32. Another useful cross-linking agent is SPDP (Pierce #21557), a heterobifunctional cross-linker for use with primary amines and sulfhydryl groups. SPDP has a molecular weight of 312.4 and a spacer arm length of 6.8 angstroms, is reactive to NHS-esters and pyridyldithio groups, and produces cleavable cross-linking such that upon further reaction, the agent is eliminated so the Hsp can be linked directly to a backbone or heterologous polypeptide. Other useful conjugating agents are SATA (Pierce #26102) for introduction of blocked SH groups for two-step cross-linking, which are deblocked with hydroxylamine-HCl (Pierce #26103), and sulfo-SMCC (Pierce #22322), reactive towards amines and sulfhydryls. Other cross-linking and coupling agents are also available from Pierce Chemical Co. (Rockford, IL). Additional compounds and processes, particularly those involving a Schiff base as an intermediate, for conjugation of proteins to other proteins or to other compositions, for example to reporter groups or to chelators for metal ion labeling of a protein, are disclosed in EP 243,929 A2 (published Nov. 4, 1987).

Polypeptides that contain carboxyl groups can be joined to lysine ε-amino groups in the heterologous polypeptide either by preformed reactive esters (such as N-hydroxy succinimide ester) or esters conjugated *in situ* by a carbodiimide-mediated reaction. The same applies to Hsps containing sulfonic acid groups, which can be transformed to sulfonyl chlorides that react with amino groups. Hsps that have carboxyl groups can be joined to amino groups on the polypeptide by an *in situ* carbodiimide method. Hsps can also be attached to hydroxyl groups of serine or threonine residues, or to sulfhydryl groups of cysteine residues.

In addition to conjugates of two polypeptides, e.g., a Hsp and a heterologous polypeptide, hybrid compounds can be constructed containing a non-peptide compound covalently linked to a polypeptide at least eight amino acids in length. The polypeptide component of this hybrid compound can be any of the heterologous polypeptides described herein as a component of a Hsp fusion protein or conjugate. Examples of the non-peptide component of this hybrid compound include polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, preferably between about 1,500 and 100 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such non-peptide compounds.

In Vitro Assays for Th1-Like Activity

Cell samples containing naive lymphocytes are prepared from any mammal, e.g., a mouse, rat, rabbit, goat, or human, and are plated at an appropriate density in one or more tissue culture plates. A naive lymphocyte is a lymphocyte that has not been exposed (either *in vivo* or *in vitro*) to the fusion protein (or to either of the polypeptides that are joined to make the fusion protein) prior to the cell's use in the *in vitro* assay. The cell sample can be derived from any of various primary or secondary lymphoid organs or tissues of an animal, e.g., spleen, lymph node, peripheral blood, bone marrow, or thymus. The sample may also be derived from any tissue in the body containing lymphoid cells, such as the lung, respiratory tract (including pharynx, larynx, trachea, bronchi, etc), and anogenital mucosa. The cell sample can include naive lymphocytes selected from NK cells, NK T cells, αβT cells and γδT cells. The cell sample can be either unfractionated or enriched for a particular cell type or cell types. In addition to naive lymphocytes, the cell sample can optionally include naive antigen presenting cells such as macrophages, dendtritic cells, and/or B cells. The cell sample can optionally include cell lines, e.g., a transformed T cell line or a T cell clone.

The cell sample is exposed *in vitro* to a fusion protein or a conjugate described herein. Following a period of incubation between the cell sample and the fusion protein or conjugate, e.g., 6, 12, 24, 36, 48, 60, 72, or 96 hours, a determination is made as to whether a Th1-like response has been elicited in the cell sample. A Th1-like response can be detected, for example, by measuring the production of particular lymphokines, e.g., IFN-gamma or TNF-beta, by the cell sample. Alternatively, a Th1-like response can be detected by assaying for cell surface marker expression, such as SLAM (signaling lymphocytic activation molecule), or for cytokine expression, using a variety of techniques (for example, flow cytometry).

In one example, pooled, unfractionated splenocyte cultures containing naive lymphocytes are prepared from a mouse and are plated in tissue culture plates. Methods of isolating and culturing splenocytes are described in Current Protocols in Immunology, Coligan et al., eds., John Wiley & Sons, 2000. Cultures of splenocytes are then exposed to different concentrations of a test protein, e.g., a recombinant Hsp fusion protein, Hsp, the antigen alone, or another antigen-containing fusion protein, for a time that is sufficient to elicit a measurable IFN-gamma response against a standard antigen-stress protein fusion protein such as, for example, HspE7, described in patent

application WO 99/07860 and employed in the Examples below. Following exposure of the cell sample to the test protein, the IFN-gamma level in the extracellular medium is determined using a suitable assay such as an IFN-gamma ELISA.

Results of the assays described below reveal that IFN-gamma release elicited by exposure of splenocytes or lymph node cells to an Hsp fusion protein is much more substantial than that induced by exposure to the antigen itself, the Hsp itself, an admixture of antigen and Hsp, or a fusion between antigen and a protein other than a Hsp.

The assay of the invention can be used to evaluate a preparation of an Hsp fusion protein (e.g., as a quality control assay) or compare different preparations of Hsp fusion proteins. The measurements taken in the assay constitute a method for identifying a particularly active batch or to eliminate substandard batches of fusion protein preparations. The assay may also be used to optimize production procedures, storage regimes, etc. In cases in which a maximal Th1-like response to a particular antigen is desired, the assays can be used to test different fusions between the antigen and different types of Hsps or Hsps of different origins. Furthermore, the assay can be used to test a series of different candidate antigens, to identify the antigen that gives rise to the most pronounced Th1-like response when fused to a Hsp.

The assay can also be used to identify regions in an antigen sequence or an Hsp sequence that are primarily responsible for eliciting a Th1-like response and thus have therapeutic potential. To identify such active regions in an antigen, fusions containing individual subregions of the antigen fused to an Hsp can be prepared and tested in the assay of the invention. To identify active regions in an Hsp, fusions containing individual subregions of the Hsp fused to the antigen can be prepared and tested. These determinations will provide the basis for the construction of shortened fusion proteins comprising subregions of antigen and/or Hsp that are sufficient to elicit a Th1-like response. Fusions containing subregions of a Hsp and/or subregions of an antigen can be tested by comparing the elicited Th1-like response to that induced by a full length fusion protein with known activity, e.g., HspE7.

The fusion proteins described herein are useful in assays for screening compounds for their effectiveness in stimulating a Th1-like response. For example, the Hsp fusion proteins that were found to stimulate IFN-gamma secretion in the *in vitro*

assay can be used as controls to test candidate compounds for their ability to produce the same effect.

The system described herein for stimulating a Th1-like response *in vitro* can be used to generate activated Th1 cells *ex vivo* for reimplantation into an individual. This may be useful for treating conditions characterized by a dominant Th2 immune response and an insufficient Th1 response.

The assay can also be used to identify compounds that can regulate a Th1-like response. Compounds can be screened for their ability to inhibit an Hsp-fusion protein-induced Th1-like response, or to promote a Th1-like response in a manner similar to a Hsp fusion protein, or to enhance the Th1-like response induced by a Hsp fusion protein (or any other protein found to act in a manner comparable to a Hsp fusion protein). Inhibitory compounds may be useful to treat conditions characterized by an inappropriate Th1 response, e.g., inflammatory and autoimmune diseases. Potential inhibitors (e.g., of binding of antigen-stress protein fusion proteins to antigen-presenting cells or of stress protein fusion-enhanced antigen processing) can be screened as follows. A cell sample comprising naive lymphocytes is mixed with a fusion protein or conjugate that is known to induce a Th1-like response, e.g., IFN-gamma secretion. Compounds to be screened as potential inhibitors are added to the cell culture either before, after, or simultaneous to the addition of the fusion protein or conjugate. The effect of the compound on the induction of a Th1-like response, e.g., as measured by IFN-gamma release, can be determined by comparing the response to that obtained when the fusion protein or conjugate alone is added to the cell sample.

In a similar manner, compounds can be screened for their ability to promote a Th1-like response. Any compound can be screened for its ability to regulate a Th1-like response, including both peptides and non-peptide chemicals. These compounds include, but are not limited to, peptides, peptidomimetics (e.g., peptoids), amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds. In this case, a cell sample comprising naive lymphocytes is contacted with a test compound. The effect of the test compound on the induction of a Th1-like response, e.g., as measured by IFN-gamma release, is then measured and compared to a control (no test sample) or compared to an Hsp fusion known to stimulate a Th1-like

response. This assay can be used to identify novel compounds that can be used to stimulate a Th1-like response. Preferably the Th1-like response stimulated by the compound is at least 25%, e.g., at least 40%, 50%, 60%, 70%, or 80%, the level of the maximum response induced by an HspE7 fusion protein. In one embodiment, the compound is preferably not a naturally occurring compound. In another embodiment, the compound is a peptide, wherein the peptide does not correspond to the fragment of a naturally occurring protein.

The following are examples of the practice of the invention. They are not to be construed as limiting the scope of the invention in any way.

Examples

Example 1: Bacterial Growth and Cell Lysis for Production of Recombinant Proteins

E. coli strains BL21(DE3) or BLR(DE3) (Novagen) were used as the host for all recombinant protein production, with the exception of pET65, which was transformed into BL21(DE3) pLysS (Novagen). BL21(DE3) pLysS cells harboring pET65 were grown in 2xYT media (20 g/L tryptone; 10 g/L yeast extract, 20 g/L NaCl; Milli-Q™ quality water) containing 30 μg/ml kanamycin and 34 μg/ml chloramphenicol, while all other transformants were grown in 2xYT media containing 30 μg/ml kanamycin. All bacterial cultures were grown in 2 L shaker flasks at 200-400 rpm to OD_{600}=0.5 and then induced with 0.5 mM IPTG for 3 hours at 37°C. Cells were then harvested by centrifugation at 4°C and 4,000 – 8,000 *g* for 5 minutes, then suspended in 300 ml of Lysis Buffer (10 mM TRIS·HCl, 10 mM 2-mercaptoethanol, pH 7.5), lysozyme was added to 200 μg/mL, and the suspension mixed and frozen at -70°C.

To purify the recombinant protein, the cells were thawed using a 37°C waterbath and proteinase inhibitors were added (2 μg/ml aprotinin, 2 μg/ml leupeptin, 2 μg/ml pepstatin and 2 mM PMSF). The cell suspension was split into 50 mL samples, stored on ice, and sonicated 3–4 times for 30 seconds at Power-Level 5 - 8 (Sonicator 450, Branson, Corp.). The supernatant was separated from the pellet by centrifugation at 35,000 – 60,000 *g* for 10 –20 minutes at 4°C. For soluble proteins, the supernatant was kept and processed as the Soluble Fraction. For proteins found in inclusion bodies,

the supernatant was discarded and the pellet was washed with Lysis Buffer (optionally containing 1 M urea, 1 %(v/v) Triton X-100). The resulting mixture was then centrifugation at 35,000 – 60,000 *g* for 10 –20 minutes at 4°C and the supernatant discarded. The pellet was dissolved in Lysis Buffer containing 8 M urea. This mixture was then centrifuged at 4°C for 10 - 20 minutes at 35,000 – 60,000 *g* and the pellet was discarded and the supernatant stored at -70°C as the Inclusion Body fraction.

Example 2: Production of Recombinant *M. bovis* BCG Hsp65 (Hsp65)

A plasmid encoding Hsp65 was constructed as follows. The *M. bovis* BCG Hsp65 coding sequence was PCR amplified from pRIB1300 (van Eden *et al.* (1988) Nature 331:171-173) using the following primers. The forward primer (w046: 5' TTC GCC ATG GCC AAG ACA ATT GCG 3'; SEQ ID NO:1) contains an ATG start codon at an NcoI site. The reverse primer (w078: 5' TTC TCG GCT AGC TCA GAA ATC CAT GCC 3'; SEQ ID NO:2) contains an Nhe I site downstream of a TGA stop codon. The PCR product was digested with NcoI and NheI, purified and ligated to pET28a (Novagen) which had been cut with NcoI and NheI. Plasmid pET65 encodes the *M. bovis* BCG Hsp65 protein, abbreviated Hsp65. The nucleotide sequence (SEQ ID NO:3) coding for expression of Hsp65 (SEQ ID NO:4) is shown in Figs. 1A-1B.

The Hsp65 protein was purified as follows. The Soluble Fraction was prepared as described above from *E. coli* BL21(DE3) pLysS cells transformed with plasmid pET65. The *M. bovis* BCG Hsp65 protein (Hsp65) present in the Soluble Fraction was purified by the following chromatographic steps: SP-Sepharose (200 ml column, Amersham Pharmacia), Q-Sepharose (200 ml column, Amersham Pharmacia), Sephacryl S-300 (500 ml column, Amersham Pharmacia) and ceramic hydroxyapatite (HAP; 100 ml column, Biorad). Purified Hsp65 was exchanged into Dulbecco's modified phosphate buffered saline (DPBS)/15% (v/v) glycerol and stored at -70°C.

Example 3: Production of Recombinant HPV16 E7 (E7)

A plasmid encoding HPV16 E7 was constructed as follows. The HPV16 E7 coding sequence was PCR-amplified from pSK/HPV16 (ATCC) using primers w280 and w134 (w280: CCA GCT GTA ACC ATG GAT GGA GAT (SEQ ID NO:5) and w134: AGC CAT GAA TTC TTA TGG TTT CTG (SEQ ID NO:6)). The PCR product was digested with restriction enzyme Nco I and EcoR I and purified from an agarose

gel. The purified PCR product was ligated to pET28a that had been previously digested with the same enzymes. The ligation reaction was used to transform *E. coli* DH5alpha and putative clones containing the HPV16 E7 gene insert were selected based on diagnostic restriction digestion. This initial restriction analysis was confirmed by DNA sequence analysis of entire gene, promoter and termination regions. DNA of the confirmed construct, named pET/E7 (NH), was then introduced by electroporation into *E. coli* strain BL21(DE3). The nucleotide sequence (SEQ ID NO:7) coding for expression of E7 (SEQ ID NO:8) is shown in Fig. 2.

The HPV16 E7 protein was purified as follows. The Soluble Fraction was prepared as described above from *E. coli* BL21(DE3) cells transformed with plasmid pET/E7 (NH). The HPV 16 E7 protein was purified by the following chromatographic steps: Q-Sepharose (100 ml column, Amersham Pharmacia); Superdex 200 (26/60 column, Amersham Pharmacia); and Ni-chelating Sepharose (100 ml, Amersham Pharmacia) under denaturing conditions with serial washings containing 2% (v/v) Triton X-100 followed by serial washing to remove residual Triton X-100, and the pooled fractions containing HPV E7 protein were then dialyzed overnight against 30 mM TRIS·HCl, 1 M NaCl, 1 mM 2-mercaptoethanol, pH 7.5. The dialyzed protein was further purified by Ni-chelating Sepharose (75 ml, Amersham Pharmacia) under denaturing conditions with serial washings containing 2% (v/v)Triton X-100 followed by serial washing to remove residual Triton X-100. The purity of the protein was checked by SDS-PAGE, the appropriate fractions pooled and dialyzed overnight at 4°C against DPBS/10 %(v/v) glycerol.

Example 4: Production of Recombinant Histidine-tagged HPV 16 E7 ((h)E7)

A plasmid encoding (h)E7 was constructed as follows. The HPV16 E7 coding sequence was PCR amplified from HPV16 genomic DNA (pSK/HPV16) using the following primers. The forward primer (w133: 5' AAC CCA GCT GCT AGC ATG CAT GGA GAT 3'; SEQ ID NO:9) contains an NheI site upstream of an ATG start codon. The reverse primer (w134: 5' AGC CAT GAA TTC TTA TGG TTT CTG 3'; SEQ ID NO:10) contains an EcoRI site downstream of a TAA stop codon. The PCR product was digested with NheI and EcoRI, purified and ligated to pET28a which had been cut with NheI and EcoRI. pET/H/E7 which encodes the HPV16 E7 protein containing an N-terminal histidine tag, abbreviated (h)E7, was used to transform *E. coli*

BL21(DE3) cells. The nucleotide sequence (SEQ ID NO:11) coding for expression of (h)E7 (SEQ ID NO:12) is shown in Fig. 3.

The (h)E7 protein was purified as follows. The Inclusion Body fraction was prepared as described above from *E. coli* BL21(DE3) cells transformed with plasmid pET/H/E7. The N-terminal histidine-tagged HPV16 E7 protein ((h)E7) present in the Inclusion Body fraction was purified using the following chromatographic steps: Ni-chelating Sepharose (60 ml, Amersham Pharmacia) under denaturing conditions with serial washings containing 2% (v/v) Triton X-100 followed by serial washing to remove residual Triton X-100. Bound (h)E7 was refolded on the resin and eluted by a 50-500 mM imidazole gradient. Purified (h)E7 was dialyzed against DPBS/25% (v/v) glycerol.

Example 5: Production of Recombinant HPV 16 E7 - *M. bovis* BCG 65 Fusion Protein (HspE7)

A plasmid encoding HspE7 was constructed as follows. The *M. bovis* BCG Hsp65 coding sequence was PCR amplified from pRIB1300 using the same forward primer (w046) as for pET65. The reverse primer (w076: 5' CGC TCG GAC GCT AGC TCA CAT ATG GAA ATC CAT GCC 3'; SEQ ID NO:13) contains an NdeI site upstream and an NheI site downstream of a TGA stop codon. The PCR product was digested with NcoI and NheI, purified and ligated to pET28a which had been cut with NcoI and NheI.

The HPV16 E7 coding sequence was PCR amplified from HPV16 genomic DNA (pSK/HPV16) using the following primers. The forward primer (w151: 5' CCA GCT GTA CAT ATG CAT GGA GAT 3'; SEQ ID NO:14) contains an ATG start codon at an NdeI site. The reverse primer (w134: 5' AGC CAT GAA TTC TTA TGG TTT CTG 3'; SEQ ID NO:15) contains an EcoRI site downstream of a TAA stop codon. The PCR product was digested with NdeI and EcoRI, purified and ligated to pET65C which had been cut with Nde I and EcoRI and the resulting plasmid (pET65C/E7-1N) was transformed into *E. coli* BL21(DE3) cells. pET65C/E7-1N encodes a fusion protein consisting of Hsp65 linked via its C-terminus to HPV16 E7, abbreviated HspE7. The nucleotide sequence (SEQ ID NO:16) coding for expression of HspE7 (SEQ ID NO:17) is shown in Figs. 4A-4B.

The HspE7 protein was purified as follows. The Soluble Fraction was prepared as described above from *E. coli* BL21(DE3) cells transformed with plasmid pET65C/E7-1N. Hsp65-HPV16 E7 fusion protein (HspE7) present in the Soluble Fraction was purified by the following chromatographic steps: 0-15% ammonium sulfate precipitation, Ni-chelating Sepharose (100 ml column, Amersham Pharmacia) and Q-Sepharose (100 ml column, Amersham Pharmacia). Endotoxin was removed by extensive washing with 1% (v/v) Triton X-100 on a Ni-chelating Sepharose column in the presence of 6M guanidine-HCl (Gu-HCl). Purified HspE7 was exchanged into DPBS/15% (v/v) glycerol and stored at -70°C.

Example 6: Production of Recombinant *M. tuberculosis* Hsp40 – HPV 16 E7 Fusion Protein (MT40-E7)

pETMT40E7 is a plasmid encoding chimeric recombinant protein MT40E7 composed of *Mycobacterium tuberculosis* (strain H37RV - ATCC 27294) hsp40 protein with hu HPV16 (ATCC 45113) E7 protein attached at the C-terminus of Hsp40. The plasmid was transformed into *E. coli* BL21(DE3) cells for protein production and purification. The nucleotide sequence (SEQ ID NO:18) coding for expression of MT40-E7 (SEQ ID NO:19) is shown in Figs. 5A-5B.

The MT40-E7 protein was purified as follows. The Inclusion Body fraction was prepared as described above from *E. coli* BL21(DE3) cells transformed with plasmid pETMT40E7. MT40-E7 protein was purified using the following chromatographic steps: Q-Sepharose (100 ml column, Amersham Pharmacia), Ni-chelating Sepharose (70 ml, Amersham Pharmacia) under native conditions with serial washings containing 2% (v/v) Triton X-100 followed by serial washing to remove residual Triton X-100. The purity of the protein was checked by SDS-PAGE, the appropriate fractions pooled and dialyzed overnight at 4°C against DPBS/25% (v/v) glycerol.

Example 7: Ovalbumin (OVA)

Ovalbumin (Lot # 37H7010) was purchased from Sigma Chemicals and purified by chromatography using 20 mL of Con A Sepharose (Amersham-Pharmacia). Fractions containing the purified product were pooled and dialyzed overnight against DPBS.

Example 8: Production of Recombinant *M. bovis* BCG Hsp65-Ovalbumin Fusion Protein (HspOva)

A plasmid encoding HspOva was constructed as follows. The full length chicken ovalbumin-coding sequence was excised from pET/OVA with Nhe I and EcoR I digestion and purified from an agarose gel. The sequence coding for expression of OVA is shown in Fig. 6. The purified product was ligated to pET65H previously digested with the same enzymes. The ligation reaction was used to transform *E. coli* DH5alpha and putative clones containing the chicken ovalbumin gene insert were selected based on diagnostic restriction digestion. This initial restriction analysis was confirmed by DNA sequence analysis of the entire fusion gene, promoter and termination regions. DNA of the confirmed construct, named pET65H/OVA, was used to transform *E. coli* BL21(DE3). The nucleotide sequence (SEQ ID NO:20) coding for expression of HspOVA (SEQ ID NO:21) is shown in Figs. 7A-7C.

The HspOva protein was purified as follows. The Inclusion Body fraction was prepared as described above from *E. coli* BL21(DE3) cells transformed with plasmid pET65H/OVA. The HspOva fusion protein present in the Inclusion Body fraction was purified using the following chromatographic steps: Q-Sepharose (100 ml column, Amersham Pharmacia) and Ni-chelating Sepharose (60 ml, Amersham Pharmacia) under denaturing conditions with serial washings containing 2% (v/v) Triton X-100 followed by serial washing to remove residual Triton X-100. The purity of the protein was checked by SDS-PAGE, the appropriate fractions pooled and dialyzed overnight at 4°C against DPBS/15% (v/v) glycerol, followed by a dialysis against DPBS/2.5 %(w/v) sucrose.

Example 9: Production of Recombinant Glutathione-S-Transferase (GST)

A plasmid encoding Gst was constructed as follows. The kanamycin resistance-coding sequence was excised from pET28a DNA with AlwN I and Xho I digestion and purified from an agarose gel. The purified product was ligated to pGEX-4T-2 that had been previously digested with the same enzymes. The ligation reaction was used to transform *E. coli* DH5alpha and putative clones containing the kanamycin resistance gene insert were selected based on diagnostic restriction digestion. This initial restriction analysis was confirmed by DNA sequence analysis of the entire insert

coding sequence, promoter and termination regions. DNA of the confirmed construct, named pGEX/K, was used to transform *E. coli* strain BL21(DE3). The nucleotide sequence (SEQ ID NO:22) coding for expression of GST (SEQ ID NO:23) is shown in Fig. 8.

The GST protein was purified as follows. The Soluble fraction was prepared as described above from *E. coli* BL21(DE3) cells transformed with plasmid pGEX/K. The GST protein present in the Soluble Fraction was purified by Glutathione-Agarose Chromatography as follows. Approximately 20 mL of Glutathione-Agarose (Sigma-Aldrich; Cat. #: G4510) was equilibrated with DPBS, and mixed and incubated overnight with the sample at room temperature on a shaker. The next morning, the resin was packed into a column and serially washed with DPBS. Endotoxin was removed by washing with 2% (v/v) Triton X-100 followed by serial washing to remove residual Triton X-100. Finally, the protein was eluted using 10 mM glutathione (reduced form), 50 mM TRIS·HCl, pH 8.0.

Example 10: Production of Recombinant Glutathione-S-Transferase – HPV 16 E7 Fusion Protein (GST-E7)

A plasmid encoding GST-E7 was constructed as follows. The HPV16 E7 coding sequence was excised from pETOVA/E7 with BamH I and EcoR I digestion and purified from an agarose gel. The purified product was ligated to pGEX/K that had been previously digested with the same enzymes. The ligation reaction was used to transform *E. coli* DH5alpha and putative clones containing the HPV16-E7 gene insert were selected based on diagnostic restriction digestion. This initial restriction analysis was confirmed by DNA sequence analysis of entire fusion gene, promoter and termination regions. DNA of the confirmed construct, named pGEX/K/E7, was used to transform *E. coli* strain BL21(DE3). The nucleotide sequence (SEQ ID NO:24) coding for expression of GST-E7 (SEQ ID NO:25) is shown in Fig. 9.

The GST-E7 protein was purified as follows. Bacteria containing the expression vector pGEX/K/E7 were grown and the protein purified using the affinity chromatography procedure essentially as described above for GST.

Example 11: Production of Recombinant HPV 16 E7 – Linker – *M. bovis* BCG Hsp65 Fusion Protein (E7-L-BCG65)

A plasmid encoding E7-L-BCG65 was constructed as follows. The HPV16 E7-coding sequence was PCR-amplified from pSK/HPV16 (ATCC) using primers w280 and w396 (w280: CCA GCT GTA ACC ATG GAT GGA GAT (SEQ ID NO:26) and w396: GCC ATG GTA CTA GTT GGT TTC TGA GAA(SEQ ID NO27:)). The PCR product was digested with restriction enzyme Nco I and Spe I and purified from an agarose gel. The purified PCR product was ligated to pET5'65 (pET5'65 is pET65 with a polyglycine linker sequence inserted at the 5' end of the *M. bovis* BCG hsp65 sequence) that had been previously digested with the same enzymes. The ligation reaction was used to transform *E. coli* DH5alpha and putative clones containing the HPV16 E7 gene insert were selected based on diagnostic restriction digestion. This initial restriction analysis was confirmed by DNA sequence analysis of entire fusion gene, promoter and termination regions. DNA of confirmed construct, named pET/E7/5'65, was used to transform *E. coli* strain BLR(DE3). The nucleotide sequence (SEQ ID NO:28) coding for expression of E7-L-BCG65 (SEQ ID NO:29) is shown in Figs. 10A-10B.

The E7-L-BCG65 protein was purified as follows. The Soluble Fraction was prepared as described above from *E. coli* BLR(DE3) cells transformed with plasmid pET/E7/5'65. The E7-L-BCG65 fusion protein present in the Soluble Fraction was purified using the following chromatographic steps: Butyl Sepharose (100 ml, Amersham-Pharmacia), Q-Sepharose (100 ml column, Amersham Pharmacia), Superdex 200 Gel Filtration (26/60 column, Amersham Pharmacia), and Ni-chelating Sepharose Fast Flow Chromotography (60 ml, Amersham Pharmacia) under denaturing conditions with serial washings containing 2% (v/v) Triton X-100 followed by serial washing to remove residual Triton X-100. The purity of the protein was checked by SDS-PAGE, the appropriate fractions pooled and dialyzed overnight at 4°C against DPBS. In order to reduce the amount of endotoxin contained in the sample, it was further purified using a pre-packed 1 ml column of DetoxiGel™ (Pierce, Rockford, IL, USA) according to the manufacturer's instructions.

Example 12: Production of Recombinant HPV 16 E7 – *M. bovis* BCG Hsp65 Fragment Fusion Protein (BCG65(F1)-E7)

A plasmid encoding BCG65(F1)-E7 was constructed as follows. The first 600 amino terminal base pairs of *M. bovis* BCG hsp65 gene were PCR-amplified from pET65C/E7-1N using primers w046 and w293 (w046: TTC GCC ATG GCC AAG ACA ATT GCG (SEQ ID NO:30) and w293: GTA CCC CGA CAT ATG GCC CTT GTC GAA CCG CAT AC(SEQ ID NO:31)). The PCR product was digested with the restriction enzymes Nco I and Nde I and purified from an agarose gel. The purified PCR product was ligated to pET65C/E7-1N that had been previously digested with the same enzymes. The ligation reaction was used to transform *E. coli* DH5alpha and putative clones containing the truncated BCG65 gene were selected based on diagnostic restriction digestion. This initial restriction analysis was confirmed by DNA sequence analysis of the entire fusion gene, promoter and termination regions. The confirmed plasmid construct, named pET65F1/E7, was used to transform *E. coli* strain BLR(DE3). The nucleotide sequence (SEQ ID NO:32) coding for expression of BCG65(F1)-E7 (SEQ ID NO:33) is shown in Fig. 11.

The BCG65(F1)-E7 protein was purified as follows. The Inclusion Body fraction was prepared as described above from *E. coli* BLR(DE3) cells transformed with plasmid pET65F1/E7. The BCG65(F1)-E7 fusion protein present in the Inclusion Body fraction was purified using the following chromatographic steps: Source 15Q Sepharose (Amersham-Pharmacia) and Ni-chelating Sepharose (60 ml, Amersham Pharmacia) under denaturing conditions with serial washings containing 2% (v/v) Triton X-100 followed by serial washing to remove residual Triton X-100. The purity of the protein was checked by SDS-PAGE, the appropriate fractions pooled and dialyzed overnight at 4°C against DPBS.

Example 13: Production of Recombinant *M. tuberculosis* Hsp10 – HPV 16 E7 Fusion Protein (TB10-E7)

Expression plasmid pETESE7 contains a chimeric gene composed of the *Mycobacterium tuberculosis* strain H37RV (ATCC 27294) groES (hsp10) coding sequence fused, at its 3' end, to the HPV16 (ATCC 45113) E7 coding. The chimeric gene was cloned into expression vector pET28a and transformed into *E. coli* BL21(DE3) cells for protein production and purification. The nucleotide sequence

(SEQ ID NO:34) coding for expression of TB10-E7 (SEQ ID NO:35) is shown in Fig. 12.

The TB10-E7 protein was purified as follows. The Inclusion Body fraction was prepared as described above from *E. coli* BL21(DE3) cells transformed with plasmid pETESE7. The TB10-E7 fusion protein present in the Inclusion Body fraction was purified using the following chromatographic steps: DEAE Sepharose (100 ml column, Amersham Pharmacia), Source 15Q Sepharose (100 ml column, Amersham Pharmacia) and Ni-chelating Sepharose (60 ml, Amersham Pharmacia) under denaturing conditions with serial washings containing 2% (v/v) Triton X-100 followed by serial washing to remove residual Triton X-100. The purity of the protein was checked by SDS-PAGE, the appropriate fractions pooled and dialyzed overnight at 4°C against DPBS/10 %(v/v) glycerol.

Example 14: Production of Recombinant HPV 16 E7 – *M. tuberculosis* Hsp71 Fusion Protein (E7-TB71)

A plasmid encoding E7-TB71 was constructed as follows. The *M. tuberculosis* hsp71 gene was PCR-amplified from clone pY3111/8 (Mehlert and Young (1989) Mol.Microbiol. 3:125-130) using primers w048 and w079 (w048: 5'-TTC ACC ATG GCT CGT GCG GTC GGG (SEQ ID NO:36) and w079: ACC TCC GCG TCC ACA GCT AGC TCA GCC(SEQ ID NO:37)). The PCR product was digested with Nco I and Nhe I, gel-purified and ligated to pET28a digested with the same enzymes to generate pET/71.

The HPV16 E7-coding sequence was PCR-amplified from pSK/HPV16 (ATCC) using primers w280 and w344 (w280: CCA GCT GTA ACC ATG GAT GGA GAT (SEQ ID NO:38) and w344: GGA TCA GAC ATG GCC ATG GCT GGT TTC TG (SEQ ID NO:39)). The PCR product was digested with restriction enzyme Nco I and purified from an agarose gel. The purified PCR product was ligated to pET/71 DNA that had been previously digested with Nco I and CIAP to remove 5' phosphate. The ligation reaction was used to transform *E. coli* DH5alpha and putative clones containing the HPV16 E7 gene insert were selected based on diagnostic restriction digestion. This initial restriction analysis was confirmed by DNA sequence analysis of entire fusion gene, promoter and termination regions. The confirmed construct, named pET/E7/71, was used to transform *E. coli* strain BL21(DE3). The nucleotide sequence

(SEQ ID NO:40) coding for expression of E7-TB71 (SEQ ID NO:41) is shown in Figs. 13A-13B. The resulting construct, pET/E7/71, was further modified (to complete sequences at the 3' end of the hsp71 gene) by replacement of a Kpn I to Nhe I fragment containing sequences from the 3' end of the hsp71 gene by a Kpn I- and Nhe I-digested PCR fragment amplified from pY3111/8 using primers w391 and w392 (w391: GAG GGT GGT TCG AAG GTA CC (SEQ ID NO:42) and w392: TTT GAT TTC GCT AGC TCA CTT GGC CTC(SEQ ID NO:43)). The resulting final plasmid, pET/E7/71', expresses HPV16 E7 fused to the amino-terminus of full-length Hsp71 protein and was used to transform *E. coli* strain BL21(DE3). The nucleotide sequence (SEQ ID NO:44) coding for expression of the fusion protein (SEQ ID NO:45) of pET/E7/71' is shown in Figs. 14A-14B.

The E7-TB71 protein was purified as follows. The Inclusion Body fraction was prepared as described above from *E. coli* BL21(DE3) cells transformed with plasmid pET/E7/71'. The E7-TB71 fusion protein present in the Inclusion Body fraction was purified using the following chromatographic steps: Q-Sepharose (100 ml column, Amersham Pharmacia) and Ni-chelating Sepharose (80 ml, Amersham Pharmacia) under native conditions with serial washings containing 2% (v/v) Triton X-100 followed by serial washing to remove residual Triton X-100. The purity of the protein was checked by SDS-PAGE, the appropriate fractions pooled and dialyzed overnight at 4°C against DPBS/10 %(v/v) glycerol.

Example 15: Production of Recombinant *Streptococcus pneumoniae* HSP65(2) – HPV 16 E7 Fusion Protein (SP65(2)-E7)

A plasmid encoding SP65(2)-E7 was constructed as follows. The *Streptococcus pneumoniae* hsp65 gene was PCR-amplified from plasmid pETP60-2 (PCT patent application WO 99/35720) using primers w384 and w385 (w384: GCA GCC CCA TGG CAA AAG AAA (SEQ ID NO:46) and w385: GCT CGA ATT CGG TCA GCT AGC TCC GCC CAT (SEQ ID NO:47)). The PCR product was digested with Nco I and EcoR I, gel-purified and ligated to pET28a digested with the same enzymes to generate pET/SP65-2C.

The HPV16 E7-coding sequence was PCR-amplified from pSK/HPV16 (ATCC) using primers w133 and w134 (w133: AAC CCA GCT GCT AGC ATG CAT GGA GAT (SEQ ID NO:48) and w134: AGC CAT GAA TTC TTA TGG TTT CTG

(SEQ ID NO:49)). The PCR product was digested with restriction enzymes Nhe I and EcoR I and purified from an agarose gel. The purified PCR product was then ligated to pET/SP65-2C that had been previously digested with Nhe I and EcoR I. The ligation reaction was used to transform *E. coli* DH5alpha and putative clones containing the HPV16 E7 insert were selected based on diagnostic restriction digestion. This initial restriction analysis was confirmed by DNA sequence analysis of entire fusion gene, promoter and termination regions. DNA of the confirmed construct, named pET/SP65c-E7, was used to transform *E. coli* strain BLR(DE3). The nucleotide sequence (SEQ ID NO:50) coding for expression of SP65(2)-E7 (SEQ ID NO:51) is shown in Figs. 15A-15B.

The SP65(2)-E7 protein was purified as follows. The Inclusion Body fraction was prepared as described above from *E. coli* BLR(DE3) cells transformed with plasmid pET/SP65c-E7. The SP65(2)-E7 fusion protein present in the Inclusion Body fraction was purified using the following chromatographic steps: Q-Sepharose (100 ml column, Amersham Pharmacia) and Ni-chelating (60 ml, Amersham Pharmacia) under denaturing conditions with serial washings containing 2% (v/v) Triton X-100 followed by serial washing to remove residual Triton X-100. The purity of the protein was checked by SDS-PAGE, the appropriate fractions pooled and dialyzed overnight at 4°C against DPBS.

Example 16: Recombinant Production of *Aspergillus fumigatus* Hsp60- HPV 16 E7 Fusion Protein (AF60-E7)

pETAF60E7 is a plasmid encoding a recombinant protein, AF60-E7, composed of the *Aspergillus fumigatus* (ATCC 26933) Hsp60 protein (without leader) (obtained as described in PCT/CA99/01152) fused at its C-terminus to the HPV16 (ATCC 45113) E7 protein sequence. Plasmid pETAF60E7 was used to transform *E. coli* BL21(DE3) cells for protein production and purification. The nucleotide sequence (SEQ ID NO:52) coding for expression of AF60-E7 (SEQ ID NO:53) is shown in Figs. 16A-16B.

The AF60-E7 protein was purified as follows. The Inclusion Body fraction was prepared as described above from *E. coli* BL21(DE3) cells transformed with plasmid pETAF60E7. AF60-E7 protein was purified using the following chromatographic steps: Source 15Q Sepharose (Amersham-Pharmacia) and Ni-chelating Sepharose (60

ml, Amersham Pharmacia) under denaturing conditions with serial washings containing 2% (v/v) Triton X-100 followed by serial washing to remove residual Triton X-100. The purity of the protein was checked by SDS-PAGE, the appropriate fractions pooled and dialyzed overnight at 4°C against DPBS.

Example 17: Stimulation of IFN-Gamma Release by a Hsp65-HPVE7 (HspE7) Fusion Protein

Pooled, unfractionated splenocytes were prepared from untreated naive C57BL/6 mice obtained from two different sources (Charles River Laboratory and Jackson Laboratory) and were plated in complete medium (complete RPMI) at 6 x 10^5 cells/well in flat bottom 96-well tissue culture plates. Replicate cultures (5) were incubated for 72 hours with 0.05 to 1.4 nmol/mL concentrations of recombinant *M. bovis* BCG Hsp65 (Hsp65), HPV16 E7 (E7) or histidine-tagged E7 ((h)E7), an admixture of *M. bovis* BCG Hsp65 and HPV16 E7 (Hsp65 + E7), or *M. bovis* BCG Hsp65 - HPV16 E7 fusion protein (HspE7). Subsequent to incubation, cells were pelleted, and supernatants were transferred to IFN-gamma capture ELISA plates.

After incubation, the replicate samples were harvested, pooled in eppendorf tubes and pelleted at 1200 rpm for 7 minutes in Beckman GS-6R centrifuge (300 x g). The supernatants were removed into cryovials and frozen at -70°C until time of analysis.

Maxisorp ELISA plates (Nunc cat# 442404A) were coated overnight at 4°C with 1 µg/mL purified rat anti-mouse IFN-gamma (PharMingen cat. no 18181D) in 0.1 M $NaHCO_3$ buffer, pH 8.2. The plates were washed with 0.05% Tween 20 in PBS then blocked with 3% BSA (albumin fraction V: Amersham cat. no 10857) in DPBS (blocking buffer) for 2 hours. After the plates were washed, recombinant mouse IFN-gamma (8000, 4000, 2000, 1000, 500, 250, 125, 62.5 pg/mL in complete RMPI) was placed in triplicate onto each ELISA plate. Sample supernatants were removed from -70°C, thawed quickly at 37°C, and placed undiluted onto the ELISA plates in duplicate. The samples were then serially diluted by seven, 3-fold dilutions in complete RPMI followed by incubation at 4°C overnight. Background ELISA values were established by measuring eight wells containing all reagents except the target antigen.

Detection of bound murine IFN-gamma was accomplished using 1 µg/mL of a rat anti-mouse IFN-gamma biotin conjugate (PharMingen cat. no 18112D) in blocking

buffer. Following washing, bound biotin-conjugated antibody was detected using a 1:1000 dilution of a streptavidin-alkaline phosphatase conjugate (Caltag cat. no SA1008). The plates were washed as before followed by the addition of a chromogenic substrate, p-nitrophenyl phosphate (pNPP; Sigma cat# N-2765) at 1 mg/mL in diethanolamine buffer, pH 9.5. After 30 minutes incubation, the color reaction was stopped using 50 µL of 100 mM EDTA, pH 8.0. The absorbance was measured at 410 nm using a Dynatech MR5000 ELISA plate reader equipped with Biolinx 2.0 software. The levels of IFN-gamma detected in test samples were extrapolated from the standard curves generated on each of the respective ELISA plates. Data is expressed as IFN-gamma released (pg/mL ± SD).

Results of assays are shown in Figs. 17A-17B. The averages from five replicates are shown along with the standard deviation. Substantial secretion of IFN-gamma was elicited by exposure of splenocytes to 0.05, 0.15, 0.46 and 1.4 nmol/mL HspE7. Hsp65 alone, E7 alone, hE7 alone, and an admixture of Hsp65 and E7 were virtually incapable of stimulating IFN-gamma release. Similar results were obtained with splenocytes prepared from mice obtained from the Charles River Laboratory (Fig. 17A) and from the Jackson Laboratory (Fig. 17B).

Example 18: Stimulation of IFN-Gamma Release by a HspE7 Fusion Protein in Splenocyte Cultures from Mice Having Different Genetic Backgrounds

Experiments similar to those presented in Example 17 were carried out using splenocytes from mice (from Jackson Laboratory) of three different haplotypes: C57BL/6 (H-2^b); Balb/c (H-2^d); and C3HeB/FeJ (H-2^k). The relative effects of the fusion protein on the different splenocyte preparations were similar, although there were differences in the absolute amounts of IFN-gamma released: the observed order being Balb/c (highest; Fig. 18A), C57BL/6 (intermediate; Fig. 18B), and C3HeB/FeJ (lowest; Fig. 18C). As in Example 17, substantially increased IFN-gamma release was induced by HspE7, but not by E7 alone, Hsp65 alone, or an admixture of E7 and Hsp65.

Example 19: Stimulation of IFN-Gamma Release by Fusion Proteins is Independent of the Nature of the Linked Antigen but Requires a Linked Stress Protein Moiety

Experiments were performed as discussed under the previous examples. It was observed that stimulation of naive splenocytes by (h)E7 or Hsp65 (*M. bovis* BCG) produced negligible IFN-gamma release, but that fusion proteins containing E7 and Hsp65 (*M. bovis* BCG) or Hsp40 (*M. tuberculosis*) substantially enhanced IFN-gamma release (Fig. 19). Virtually no induction of IFN-gamma release was mediated by a fusion protein containing E7 and glutathione-S-transferase (GST). When a fusion protein including an ovalbumin fragment and an Hsp (*M. bovis* BCG Hsp65) was tested, high levels of IFN-gamma release were detected. The IFN-gamma release mediated by the HspOVA fusion protein exceeded that resulting from addition of OVA alone to the cell culture. These results demonstrate that the induced release of IFN-gamma is not dependent on the presence of the E7 antigen in the fusion protein, but that other antigens fused to an Hsp can similarly enhance IFN-gamma production.

Example 20: Stimulation of IFN-Gamma Release by E7 Fusion Proteins Having Different Stress Protein Moieties

Experiments were performed as discussed under the previous examples. HPV16 E7 was fused to different Hsps, i.e., *M. tuberculosis* Hsp10 (TB10-E7), *M. bovis* BCG Hsp65 (HspE7), *Streptococcus pneumoniae* Hsp65 (2) (SP65(2)-E7), and *Aspergillus fumigatus* Hsp60 (AF60-E7). Furthermore, in two cases (E7-L-BCG65 and E7-TB71) the Hsp (*M. bovis* BCG Hsp65 and *M. tuberculosis* Hsp71, respectively) was added to the carboxy terminus of the E7 antigen instead of to the amino terminus as in the other fusions.

Additionally, one construct was tested, in which the E7 antigen was linked to the amino terminal one third (residues 1-200) of the *M. bovis* BCG Hsp65 sequence (BCG65(F1)-E7), rather than an intact Hsp. It was observed (Figs. 20A-20B) that stimulation of IFN-gamma release occurred upon exposure of splenocytes to all the different fusion proteins, although differences in the magnitude of the responses were noted. Thus, fusions containing different Hsps, including Hsp65 from different organisms as well as different types of Hsps, were capable of eliciting enhanced IFN-gamma release. Furthermore, fusions containing a stress protein at either the amino terminal end or at the carboxy terminal end of the E7 antigen were active. Finally,

BCG65(F1)-E7, containing amino acids 1-200 of *M. bovis* BCG Hsp65, induced IFN-gamma secretion in a manner similar to the full-length Hsp65 sequence (HspE7).

Example 21: Stimulation of IFN-Gamma Release by HspE7 Fusion Protein in Lymph Node Cell Cultures

To test for their ability to induce IFN-gamma release, various concentrations of the HspE7 proteins (diluted to the desired starting concentration in complete medium, defined as RPMI 1640 with 10% fetal calf serum) were added as replicate samples (3 to 5 replicates) to flat bottom 96-well tissue culture plates. For the cellular component of the assay, three inguinal lymph nodes were aseptically removed from untreated C57BL/6 mice and placed in 5 ml of Hank's balanced salt solution supplemented with 5% fetal calf serum (medium). Following their transfer to a sterile 0.22 micron nylon mesh, a sterile syringe plunger was used to disperse the cells through the mesh. Medium was used to rinse the cells, yielding a pooled, unfractionated single cell suspension. Cells were washed once, resuspended in complete medium and added to wells at 6×10^5 cells/well, to a final volume of 0.2 ml. Cultures were exposed to the HspE7 protein in medium or to medium alone for 72 hours at 37^0C in a 5% CO_2 atmosphere. Following incubation, replicate cultures were pooled, cells pelleted by centrifugation and supernatants either measured for IFN-gamma content by ELISA according to the procedure described in Example 17, or frozen immediately at -70^0C for later analysis.

Fig. 21 shows the results of the above experiment, comparing induction of IFN-gamma release by lymph node cells and by splenocytes. The fusion protein was found to elicit a release of IFN-gamma in both cell types. The IFN-gamma release elicited by the fusion protein greatly exceeded that induced by Hsp65 alone.

Example 22: Regression of Pre-Established Tumors *in vivo* Induced by Administration of Hsp Fusion Proteins

Human papilloma virus type 16 (HPV16) is an infectious agent associated with the induction of cervical cancer and its premalignant precursor, cervical intraepithelial neoplasia. The following experiments use Hsp - HPV16 E7 fusion proteins of the invention to target immune recognition as part of a strategy to eliminate HPV16 E7-expressing host cells.

The H-2^b murine epithelial cell-derived tumor line, TC-1 (co-transformed with HPV16 E6 and E7 and activated human Ha-*ras*), was obtained from T.C. Wu of Johns Hopkins University (Baltimore, MD). The use of TC-1 cells in assays similar to those used herein is described in PCT patent application WO 99/07860. TC-1 was maintained in complete medium, consisting of: RPMI 1640 (ICN, cat no. 1260354) supplemented with 10% FBS (Hyclone, cat no. SH30071); 2 mM L-Glutamine (ICN, cat no. 16-801-49); 10 mM HEPES (ICN, cat no. 16-884-49); 0.1mM MEM Non Essential Amino Acid Solution (Gibco BRL, cat no. 11140-050); 1 mM MEM Sodium Pyruvate (Gibco BRL, cat no. 11360-070); 50 μM 2-Mercaptoethanol (Sigma, cat no. M-7522); and 50 mcg/mL Gentamycin Sulfate (Gibco BRL, cat no. 15750-011). The medium was also supplemented with G418 (0.4 mg/mL active, Gibco BRL, cat no. 11811-023) and Hygromycin B (0.2 mg/mL active, Calbiochem, cat no. 400051).

Since the TC-1 cell line was derived from a C57BL/6 mouse, this mouse strain was used as the host in these experiments. Female C57BL/6 mice of approximately 8 to 10 weeks of age were purchased from Charles River Canada (St-Constant, Quebec, Canada) and housed using filter top cages (four animals per cage).

TC-1 cells were prepared for implantation as follows. TC-1 cells were seeded at a density of 2 - 5x10^4 cells /mL and incubated for two to four days until 70 to 90% confluent. Cells were trypsinized using a 30 second exposure to 0.25% Trypsin (10x stock, Gibco cat. no. 1505-065, diluted to 1x with DPBS), then diluted four-fold with supplemented complete medium. Following trypsinization, TC-1 cells were pelleted at 4°C at 1000 rpm (250x g) for 4 minutes, the supernatant removed by aspiration and 30 mL of cold DPBS added. The cells were then pelleted at 4°C at 700 rpm (100x g) for 4 minutes, the supernatant removed by aspiration, and a minimal amount (approx. 5 mL) of cold DPBS added. The final cell density for injection was adjusted to 6.5 x 10^5 viable cells per mL, as measured by the trypan blue dye exclusion method. At least 90% of the cells used for TC-1 inoculations were viable. The cells were stored on ice for immediate injection into mice.

TC-1 cells were implanted as follows. Between 24 to 72 hours prior to implantation, the hind flank of each mouse was shaved. TC-1 cells were prepared as described above and held on ice until injected. All injections were performed within two hours of cell trypsinization. The cells were swirled gently in the centrifuge tube and drawn into a 1 mL syringe (Becton-Dickinson, cat. no. 309602) without a needle.

A 25 gauge needle (Becton-Dickinson, cat. no. 305122) was then attached and any air bubbles were expelled. The shaved skin was raised gently and the needle was inserted bevel side up just beneath the skin surface. Cells (1.3×10^5) were injected in a 0.2 mL volume for all studies. A fresh syringe and needle was used for every fifth injection.

Fusion proteins were injected as follows. On treatment days, the fusion proteins HspE7, SP65(2)-E7, AF60-E7, E7-TB71 (shown if Figs. 23A and 23B as E7-MT71), MT40-E7 and TB10-E7 (prepared as described above) were removed from –70°C storage and thawed in a 37°C water bath. Dulbecco's phosphate buffered saline (DPBS) (4°C) was added to obtain the protein concentration desired for injection. The diluted fusion protein was held on ice until drawn into a 1 mL syringe (Becton-Dickinson, cat no. 309602) with a 30 gauge needle (Becton-Dickinson, cat no. 3095106). The same syringe was used to inject 0.2 mL of fusion protein into each mouse within a dose group; the syringe was refitted with a fresh needle for every fifth injection. Mice were injected subcutaneously in the scruff of the neck, as high on the neck as possible.

Tumor incidence (TI) was measured as follows. TI was generally recorded three times per week, beginning eight days after tumor implantation and continuing for eight weeks. Mice were assessed for the presence or absence of subcutaneous tumor by palpation and visual observation of the tumor injection site.

Tumor volume was measured as follows. Volumes of palpable subcutaneous tumor nodules were measured beginning on approximately Day 8 post implantation. The two longest orthogonal dimensions were measured using a Fowler Sylvac Ultra-Cal Mark III digital caliper with computerized data collection. Data points were tabulated in a Microsoft Excel spreadsheet. Tumor nodule measurements were extrapolated to mm^3 using the formula $V = W^2 \times L \times 0.5$ (where V represents volume, W represents width and L represents length) and are presented as average tumor volume ± standard error of the mean. The Student's t test function of Excel (two-tailed, unpaired samples, equal variances) was used to test the significance ($p < 0.05$) of the difference of the means of tumor volumes in each group.

Seven different HPV16 E7 fusion proteins linked to various hsps were tested for their ability to regress a tumor *in vivo*.

In the first experiment, C57BL/6 mice (18 per group) were inoculated subcutaneously with 1.3×10^5 TC-1 cells in the right hind flank (Day 0). After 7 days,

groups of mice were treated with 0.2 mL of either DPBS (saline), 115 ug HspE7, 100 ug SP65(2)-E7, or 100 ug AF60-E7. The doses of the two latter proteins were chosen based on the same molar equivalent of E7 contained in HspE7. The mice were monitored for the presence or absence of tumor in addition to tumor volume. The data are represented as percent tumor incidence (TI) per group (Fig. 22A) and tumor volume, expressed as average tumor volume ± standard error of the mean (Fig. 22B).

As indicated in Fig. 22A, the majority of animals had detectable tumor by Day 8 post implantation and by Day 13 tumor was evident in 94 to 100% of the mice. After this timepoint, TI in all of the mice declined until day 25 when the incidence for the DPBS-treated animals stabilized to approximately 50% for the remainder of the observation period. In contrast, the animals treated with fusion proteins showed a comparatively sharp decline in TI until day 28, when none of the animals had detectable tumor. This complete absence of tumor was observed for the remainder of the observation period for most of these animals. The complete regression of tumor in the animals treated with the fusion proteins was also clearly seen when measured by tumor volume. Figure 22B shows that by day 28, the average tumor volume of the animals treated with the fusion proteins was not detectable. By comparison, the average tumor volume of those animals treated with DPBS rose steadily from day 25 onwards.

In the second experiment, C57BL/6 mice (18 per group) were inoculated subcutaneously with 1.3×10^5 TC-1 cells in the right hind flank (Day 0). After 7 days, groups of mice were treated with 0.2 mL of either DPBS (saline), 100 ug HspE7, 100 ug MT40-E7, 100 ug E7-TB71 (shown if Figs. 23A and 23B as E7-MT71), or 100 ug TB10-E7. The mice were monitored for the presence or absence of tumor in addition to tumor volume. The data are represented as percent tumor incidence (TI) per group (Fig. 23A) and tumor volume, expressed as average tumor volume ± standard error of the mean (Fig. 23 B).

As in Figure 22A, a majority (approximately 95%) of the animals had visible and palpable tumors on day 8 post tumor implantation (Fig. 23A). By day 19, a decrease in TI was apparent. Following this, a sharp decrease in TI for all of the fusion protein-treated animals was observed such that by day 33, practically all of the animals were tumor-free. In contrast, the TI of the mice treated with DPBS had stabilized to approximately 75%. Fig. 23B shows the average tumor volumes of the mice treated

with the respective fusion proteins. The decrease in TI was reflected by the marked decrease in tumor volumes. Average tumor volumes for the animals treated with any of the fusion proteins was essentially not measurable by day 30.

What is claimed is:

1. A method of determining whether a fusion protein stimulates a Th1-like response, the method comprising:

(a) providing a cell sample comprising naive lymphocytes *in vitro*;

(b) providing a fusion protein comprising (i) a heat shock protein (Hsp) or a fragment thereof at least eight amino acid residues in length, fused to (ii) a heterologous polypeptide at least eight amino acid residues in length;

(c) contacting the cell sample with the fusion protein; and

(d) determining whether the fusion protein stimulates a Th1-like response in the cell sample.

2. The method of claim 1, wherein the Hsp is selected from the group consisting of Hsp65, Hsp40, Hsp10, Hsp60, and Hsp71.

3. The method of claim 2, wherein the fusion protein comprises a polypeptide selected from the group consisting of Hsp65, Hsp40, Hsp10, Hsp60, and Hsp71.

4. The method of claim 1, wherein the fusion protein comprises amino acids 1-200 of Hsp65 of *Mycobacterium bovis*.

5. The method of claim 1, wherein the heterologous polypeptide comprises a sequence identical to at least eight consecutive amino acids of (i) a protein of a human pathogen or (ii) a tumor associated antigen.

6. The method of claim 1, wherein the heterologous polypeptide comprises a sequence identical to at least eight consecutive amino acids of a protein of a human virus.

7. The method of claim 6, wherein the virus is selected from the group consisting of human papilloma virus (HPV), herpes simplex virus (HSV), hepatitis B

virus (HBV), hepatitis C virus (HCV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), influenza virus, measles virus, and human immunodeficiency virus (HIV).

8. The method of claim 7, wherein the heterologous polypeptide comprises HPV E6.

9. The method of claim 7, wherein the heterologous polypeptide comprises HPV E7.

10. The method of claim 1, wherein the heterologous polypeptide comprises HPV 16 E7 or a fragment thereof at least eight amino acid residues in length.

11. The method of claim 1, wherein the heterologous polypeptide comprises HPV 16 E6 or a fragment thereof at least eight amino acid residues in length.

12. The method of claim 10, wherein the fusion protein comprises *Mycobacterium bovis* Hsp65 and HPV 16 E7.

13. The method of claim 1, wherein the cell sample comprises cells derived from a spleen, lymph node, peripheral blood, bone marrow, thymus, lung, respiratory tract, or anogenital mucosa

14. The method of claim 1, wherein the cell sample comprises splenocytes or lymph node cells.

15. The method of claim 1, wherein the detecting step comprises detecting IFN-gamma produced by the cell sample.

16. The method of claim 1, comprising the further steps of

(e) providing a second cell sample comprising naive lymphocytes;

(f) contacting the second cell sample with a second fusion protein; and

(g) determining whether the second fusion protein stimulates a Th1-like response in the second cell sample,

wherein the first fusion protein comprises the sequence of a full-length, naturally occurring Hsp, and the second fusion protein comprises at least eight amino acids but less than all of the sequence of a naturally occurring Hsp.

17. A method of screening a compound, the method comprising:

(a) providing a cell sample comprising naive lymphocytes *in vitro*;

(b) providing a fusion protein comprising (i) a Hsp or a fragment thereof at least eight amino acid residues in length, fused to (ii) a heterologous polypeptide at least eight amino acid residues in length;

(c) contacting the cell sample with the compound and the fusion protein; and

(d) determining whether the cell sample exhibits a Th1-like response following the contacting step,

wherein a decrease in the Th1-like response in the presence of the compound compared to in the absence of the compound indicates that the compound inhibits a Th1-like response by the cell sample.

18. The method of claim 17, wherein the determining step comprises detecting IFN-gamma produced by the cell sample.

19. The method of claim 17, wherein the cell sample comprises cells derived from a spleen, lymph node, peripheral blood, bone marrow, thymus, lung, respiratory tract, or anogenital mucosa

20. The method of claim 17, wherein the cell sample comprises splenocytes or lymph node cells.

21. The method of claim 17, wherein the Hsp is selected from the group consisting of Hsp65, Hsp40, Hsp10, Hsp60, and Hsp71.

22. The method of claim 21, wherein the fusion protein comprises a polypeptide selected from the group consisting of Hsp65, Hsp40, Hsp10, Hsp60, and Hsp71.

23. The method of claim 17, wherein the heterologous polypeptide comprises HPV E6.

24. The method of claim 17, wherein the heterologous polypeptide comprises HPV E7.

25. The method of claim 17, wherein the fusion protein comprises *Mycobacterium bovis* Hsp65 and HPV 16 E7.

26. A method of screening a compound, the method comprising:

(a) providing a cell sample comprising naive lymphocytes *in vitro*;

(b) providing a fusion protein comprising (i) a Hsp or a fragment thereof at least eight amino acid residues in length, fused to (ii) a heterologous polypeptide at least eight amino acid residues in length;

(c) contacting the cell sample with the compound and the fusion protein; and

(d) determining whether the cell sample exhibits a Th1-like response following the contacting step,

wherein an increase in the Th1-like response in the presence of the compound compared to in the absence of the compound indicates that the compound promotes a Th1-like response by the cell sample.

27. The method of claim 26, wherein the determining step comprises detecting IFN-gamma produced by the cell sample.

28. The method of claim 26, wherein the cell sample comprises cells derived from a spleen, lymph node, peripheral blood, bone marrow, thymus, lung, respiratory tract, or anogenital mucosa

29. The method of claim 26, wherein the cell sample comprises splenocytes or lymph node cells.

30. The method of claim 26, wherein the Hsp is selected from the group consisting of Hsp65, Hsp40, Hsp10, Hsp60, and Hsp71.

31. The method of claim 30, wherein the fusion protein comprises a polypeptide selected from the group consisting of Hsp65, Hsp40, Hsp10, Hsp60, and Hsp71.

32. The method of claim 26, wherein the heterologous polypeptide comprises HPV E6.

33. The method of claim 26, wherein the heterologous polypeptide comprises HPV E7.

34. The method of claim 26, wherein the fusion protein comprises *Mycobacterium bovis* BCG Hsp65 and HPV 16 E7.

35. A method of determining whether a hybrid compound stimulates a Th1-like response, the method comprising:

(a) providing a cell sample comprising naive lymphocytes *in vitro*;

(b) providing a hybrid compound that is non-naturally occurring and comprises (i) a non-peptide compound having a molecular weight of less than 1,500, covalently linked to (ii) a polypeptide of at least eight amino acids in length, wherein the hybrid compound is made by covalently linking the non-peptide compound to the polypeptide;

(c) contacting the cell sample with the hybrid compound; and

(d) determining whether the hybrid compound stimulates a Th1-like response in the cell sample.

36. The method of claim 35, wherein the non-peptide compound has a molecular weight of at least 100.

37. A method of determining whether a hybrid compound stimulates a Th1-like response, the method comprising:

(a) producing a hybrid compound by covalently linking a non-peptide compound to a polypeptide of at least eight amino acids in length;

(b) providing a cell sample comprising naive lymphocytes *in vitro*;

(c) contacting the cell sample with the hybrid compound; and

(d) determining whether the hybrid compound stimulates a Th1-like response in the cell sample.

38. The method of claim 37, wherein the non-peptide compound has a molecular weight between 100 and 1,500.

39. A method of determining whether a fusion protein stimulates a Th1-like response, the method comprising:

(a) providing a cell sample comprising naive lymphocytes *in vitro*;

(b) providing a fusion protein comprising (i) a first polypeptide at least eight amino acids in length, fused to (ii) a second polypeptide at least eight amino acids in length;

(c) contacting the cell sample with the fusion protein; and

(d) detecting a Th1-like response exhibited by the cell sample following the contacting step.

40. The method of claim 39, wherein the detected Th1-like response is greater than a Th1-like response exhibited by a second cell sample comprising naive lymphocytes when the second cell sample is contacted with either the first polypeptide, the second polypeptide, or a mixture of the first polypeptide and the second polypeptide.

41. The method of claim 40, wherein the detected Th1-like response is at least two times greater than the Th1-like response exhibited by the second cell sample.

42. The method of claim 40, wherein the detected Th1-like response is at least five times greater than the Th1-like response exhibited by the second cell sample.

43. A fusion protein comprising (i) a Hsp10 protein or a fragment thereof at least eight amino acid residues in length, and (ii) a heterologous polypeptide at least eight amino acids in length.

44. The fusion protein of claim 43, comprising a Hsp10 protein.

45. The fusion protein of claim 44, wherein the Hsp10 protein is a mycobacterial protein.

46. The fusion protein of claim 45, comprising the *Mycobacterium tuberculosis* Hsp10 protein.

47. The fusion protein of claim 43, wherein the heterologous polypeptide comprises a sequence identical to at least eight consecutive amino acids of a protein of a human virus.

48. The fusion protein of claim 47, wherein the human virus is HPV.

49. The fusion protein of claim 48, wherein the heterologous polypeptide comprises HPV16 E7.

50. A fusion protein comprising (i) a Hsp40 protein or a fragment thereof at least eight amino acid residues in length, and (ii) a heterologous polypeptide at least eight amino acids in length.

51. The fusion protein of claim 50, comprising a Hsp40 protein.

52. The fusion protein of claim 51, wherein the Hsp40 protein is a mycobacterial protein.

53. The fusion protein of claim 52, comprising the *Mycobacterium tuberculosis* Hsp40 protein.

54. The fusion protein of claim 50, wherein the heterologous polypeptide comprises a sequence identical to at least eight consecutive amino acids of a protein of a human virus.

55. The fusion protein of claim 54, wherein the human virus is HPV.

56. The fusion protein of claim 55, wherein the heterologous polypeptide comprises HPV16 E7.

57. A fusion protein comprising (i) a Hsp71 protein or a fragment thereof at least eight amino acid residues in length, and (ii) a heterologous polypeptide at least eight amino acids in length.

58. The fusion protein of claim 57, comprising a Hsp71 protein.

59. The fusion protein of claim 58, wherein the Hsp71 protein is a mycobacterial protein.

60. The fusion protein of claim 59, comprising the *Mycobacterium tuberculosis* Hsp71 protein.

61. The fusion protein of claim 57, wherein the heterologous polypeptide comprises a sequence identical to at least eight consecutive amino acids of a protein of a human virus.

62. The fusion protein of claim 61, wherein the human virus is HPV.

63. The fusion protein of claim 62, wherein the heterologous polypeptide comprises HPV16 E7.

64. A method of determining whether a compound stimulates a Th1-like response, the method comprising:

(a) providing a cell sample comprising naive lymphocytes *in vitro*;

(b) providing a compound;

(c) contacting the cell sample with the compound; and

(d) detecting a Th1-like response exhibited by the cell sample following the contacting step.

5

```
3/1                                                                 33/11
atg gCC AAG ACA ATT GCG TAC GAC GAA GAG GCC CGT CGC GGC CTC GAG CGG GGC TTG AAC
M   A   K   T   I   A   Y   D   E   E   A   R   R   G   L   E   R   G   L   N
63/21                                                               93/31
GCC CTC GCC GAT GCG GTA AAG GTG ACA TTG GGC CCC AAG GGC CGC AAC GTC GTC CTG GAA
A   L   A   D   A   V   K   V   T   L   G   P   K   G   R   N   V   V   L   E
123/41                                                              153/51
AAG AAG TGG GGT GCC CCC ACG ATC ACC AAC GAT GGT GTG TCC ATC GCC AAG GAG ATC GAG
K   K   W   G   A   P   T   I   T   N   D   G   V   S   I   A   K   E   I   E
183/61                                                              213/71
CTG GAG GAT CCG TAC GAG aag atc ggc GCC GAG CTG GTC AAA GAG GTA GCC AAG AAG ACC
L   E   D   P   Y   E   K   I   G   A   E   L   V   K   E   V   A   K   K   T
243/81                                                              273/91
GAT GAC GTC GCC GGT GAC GGC ACC ACG ACG GCC ACC GTG CTG GCC CAG GCG TTG GTT CGC
D   D   V   A   G   D   G   T   T   T   A   T   V   L   A   Q   A   L   V   R
303/101                                                             333/111
gAg GGC CTG CGC AAC GTC GCG GCC GGC GCC AAC CCG CTC GGT CTC AAA CGC GGC ATC GAA
E   G   L   R   N   V   A   A   G   A   N   P   L   G   L   K   R   G   I   E
363/121                                                             393/131
AAG GCC GTG GAG AAG GTC ACC GAG ACC CTG CTC AAG GGC GCC AAG GAG GTC GAG ACC AAG
K   A   V   E   K   V   T   E   T   L   L   K   G   A   K   E   V   E   T   K
423/141                                                             453/151
GAG CAG ATT GCG GCC ACC GCA GCG ATT TCG GCG GGT GAC CAG TCC ATC GGT GAC CTG ATC
E   Q   I   A   A   T   A   A   I   S   A   G   D   Q   S   I   G   D   L   I
483/161                                                             513/171
GCC GAG GCG ATG GAC AAG GTG GGC AAC GAG GGC GTC ATC ACC GTC GAG GAG TCC AAC ACC
A   E   A   M   D   K   V   G   N   E   G   V   I   T   V   E   E   S   N   T
543/181                                                             573/191
TTT GGG CTG CAG CTC GAG CTC ACC GAG GGT ATG CGG TTC GAC AAG GGC TAC ATC TCG GGG
F   G   L   Q   L   E   L   T   E   G   M   R   F   D   K   G   Y   I   S   G
603/201                                                             633/211
TAC TTC GTG ACC GAC CCG GAG CGT CAG GAG GCG GTC CTG GAG GAC CCC TAC ATC CTG CTG
Y   F   V   T   D   P   E   R   Q   E   A   V   L   E   D   P   Y   I   L   L
663/221                                                             693/231
GTC AGC TCC AAG GTG TCC ACT GTC AAG GAT CTG CTG CCG CTG CTC GAG AAG GTC ATC GGA
V   S   S   K   V   S   T   V   K   D   L   L   P   L   L   E   K   V   I   G
723/241                                                             753/251
GCC GGT AAG CCG CTG CTG ATC ATC GCC GAG GAC GTC GAG GGC GAG GCG CTG TCC ACC CTG
A   G   K   P   L   L   I   I   A   E   D   V   E   G   E   A   L   S   T   L
783/261                                                             813/271
GTC GTC AAC AAG ATC CGC GGC ACC TTC AAG TCG GTG GCG GTC AAG GCT CCC GGC TTC GGC
V   V   N   K   I   R   G   T   F   K   S   V   A   V   K   A   P   G   F   G
843/281                                                             873/291
GAC CGC CGC AAG GCG ATG CTG CAG GAT ATG GCC ATT CTC ACC GGT GGT CAG GTG ATC AGC
D   R   R   K   A   M   L   Q   D   M   A   I   L   T   G   G   Q   V   I   S
903/301                                                             933/311
GAA GAG GTC GGC CTG ACG CTG GAG AAC GCC GAC CTG TCG CTG CTA GGC AAG GCC CGC AAG
E   E   V   G   L   T   L   E   N   A   D   L   S   L   L   G   K   A   R   K
963/321                                                             993/331
GTC GTG GTC ACC AAG GAC GAG ACC ACC ATC GTC GAG GGC GCC GGT GAC ACC GAC GCC ATC
V   V   V   T   K   D   E   T   T   I   V   E   G   A   G   D   T   D   A   I
1023/341                                                            1053/351
GCC GGA CGA GTG GCC CAG ATC CGC CAG GAG ATC GAG AAC AGC GAC TCC GAC TAC GAC CGT
A   G   R   V   A   Q   I   R   Q   E   I   E   N   S   D   S   D   Y   D   R
1083/361                                                            1113/371
GAG AAG CTG CAG GAG CGG CTG GCC AAG CTG GCC GGT GGT GTC GCG GTG ATC AAG GCC GGT
E   K   L   Q   E   R   L   A   K   L   A   G   G   V   A   V   I   K   A   G
1143/381                                                            1173/391
GCC GCC ACC GAG GTC GAA CTC AAG GAG CGC AAG CAC CGC ATC GAG GAT GCG GTT CGC AAT
A   A   T   E   V   E   L   K   E   R   K   H   R   I   E   D   A   V   R   N
1203/401                                                            1233/411
GCC AAG GCC GCC GTC GAG GAG GGC ATC GTC GCC GGT GGG GGT GTG ACG CTG TTG CAA GCG
A   K   A   A   V   E   E   G   I   V   A   G   G   G   V   T   L   L   Q   A
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FIG. 1A

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1263/421                                                1293/431
GCC CCG ACC CTG GAC GAG CTG AAG CTC GAA GGC GAC GAG GCG ACC GGC GCC AAC ATC GTG
A   P   T   L   D   E   L   K   L   E   G   D   E   A   T   G   A   N   I   V
1323/441                                                1353/451
AAG GTG GCG CTG GAG GCC CCG CTG AAG CAG ATC GCC TTC AAC TCC GGG CTG GAG CCG GGC
K   V   A   L   E   A   P   L   K   Q   I   A   F   N   S   G   L   E   P   G
1383/461                                                1413/471
GTG GTG GCC GAG AAG GTG CGC AAC CTG CCG GCT GGC CAC GGA CTG AAC GCT CAG ACC GGT
V   V   A   E   K   V   R   N   L   P   A   G   H   G   L   N   A   Q   T   G
1443/481                                                1473/491
GTC TAC GAG GAT CTG CTC GCT GCC GGC GTT GCT GAC CCG GTC AAG GTG ACC CGT TCG GCG
V   Y   E   D   L   L   A   A   G   V   A   D   P   V   K   V   T   R   S   A
1503/501                                                1533/511
CTG CAG AAT GCG GCG TCC ATC GCG GGG CTG TTC CTG ACC ACC GAG GCC GTC GTT GCC GAC
L   Q   N   A   A   S   I   A   G   L   F   L   T   T   E   A   V   V   A   D
1563/521                                                1593/531
AAG CCG GAA AAG GAG AAG GCT TCC GTT CCC GGT GGC GGC GAC ATG GGT GGC ATG GAT TTC
K   P   E   K   E   K   A   S   V   P   G   G   G   D   M   G   G   M   D   F
1623/541
TGA
*
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FIG. 1B

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3/1                                                 33/11
ATG gAT GGA GAT ACA CCT ACA TTG CAT GAA TAT ATG TTA GAT TTG CAA CCA GAG ACA ACT
M   D   G   D   T   P   T   L   H   E   Y   M   L   D   L   Q   P   E   T   T
63/21                                               93/31
GAT CTC TAC TGT TAT GAG CAA TTA AAT GAC AGC TCA GAG GAG GAG GAT GAA ATA GAT GGT
D   L   Y   C   Y   E   Q   L   N   D   S   S   E   E   E   D   E   I   D   G
123/41                                              153/51
CCA GCT GGA CAA GCA GAA CCG GAC AGA GCC CAT TAC AAT ATT GTA ACC TTT TGT TGC AAG
P   A   G   Q   A   E   P   D   R   A   H   Y   N   I   V   T   F   C   C   K
183/61                                              213/71
TGT GAC TCT ACG CTT CGG TTG TGC GTA CAA AGC ACA CAC GTA GAC ATT CGT ACT TTG GAA
C   D   S   T   L   R   L   C   V   Q   S   T   H   V   D   I   R   T   L   E
243/81                                              273/91
GAC CTG TTA ATG GGC ACA CTA GGA ATT GTG TGC CCC ATC TGT TCT CAG AAA CCA TAA
D   L   L   M   G   T   L   G   I   V   C   P   I   C   S   Q   K   P   *
```

FIG. 2

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3/1                                                 33/11
ATG GGC AGC AGC CAT CAT CAT CAT CAT CAC AGC AGC GGC CTG GTG CCG CGC GGC AGC CAT
M   G   S   S   H   H   H   H   H   H   S   S   G   L   V   P   R   G   S   H
63/21                                               93/31
ATG gct agc ATG CAT GGA GAT ACA CCT ACA TTG CAT GAA TAT ATG TTA GAT TTG CAA CCA
M   A   S   M   H   G   D   T   P   T   L   H   E   Y   M   L   D   L   Q   P
123/41                                              153/51
GAG ACA ACT GAT CTC TAC TGT TAT GAG CAA TTA AAT GAC AGC TCA GAG GAG GAG GAT GAA
E   T   T   D   L   Y   C   Y   E   Q   L   N   D   S   S   E   E   E   D   E
183/61                                              213/71
ATA GAT GGT CCA GCT GGA CAA GCA GAA CCG GAC AGA GCC CAT TAC AAT ATT GTA ACC TTT
I   D   G   P   A   G   Q   A   E   P   D   R   A   H   Y   N   I   V   T   F
243/81                                              273/91
TGT TGC AAG TGT GAC TCT ACG CTT CGG TTG TGC GTA CAA AGC ACA CAC GTA GAC ATT CGT
C   C   K   C   D   S   T   L   R   L   C   V   Q   S   T   H   V   D   I   R
303/101                                             333/111
ACT TTG GAA GAC CTG TTA ATG GGC ACA CTA GGA ATT GTG TGC CCC ATC TGT TCT CAG AAA
T   L   E   D   L   L   M   G   T   L   G   I   V   C   P   I   C   S   Q   K
363/121
CCA TAA
P   *
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FIG. 3

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3/1                                                                 33/11
atg gCC AAG ACA ATT GCG TAC GAC GAA GAG GCC CGT CGC GGC CTC GAG CGG GGC TTG AAC
M   A   K   T   I   A   Y   D   E   E   A   R   R   G   L   E   R   G   L   N
63/21                                                               93/31
GCC CTC GCC GAT GCG GTA AAG GTG ACA TTG GGC CCC AAG GGC CGC AAC GTC GTC CTG GAA
A   L   A   D   A   V   K   V   T   L   G   P   K   G   R   N   V   V   L   E
123/41                                                              153/51
AAG AAG TGG GGT GCC CCC ACG ATC ACC AAC GAT GGT GTG TCC ATC GCC AAG GAG ATC GAG
K   K   W   G   A   P   T   I   T   N   D   G   V   S   I   A   K   E   I   E
183/61                                                              213/71
CTG GAG GAT CCG TAC GAG aag atc ggc GCC GAG CTG GTC AAA GAG GTA GCC AAG AAG ACC
L   E   D   P   Y   E   K   I   G   A   E   L   V   K   E   V   A   K   K   T
243/81                                                              273/91
GAT GAC GTC GCC GGT GAC GGC ACC ACG ACG GCC ACC GTG CTG GCC CAG GCG TTG GTT CGC
D   D   V   A   G   D   G   T   T   T   A   T   V   L   A   Q   A   L   V   R
303/101                                                             333/111
gAg GGC CTG CGC AAC GTC GCG GCC GGC GCC AAC CCG CTC GGT CTC AAA CGC GGC ATC GAA
E   G   L   R   N   V   A   A   G   A   N   P   L   G   L   K   R   G   I   E
363/121                                                             393/131
AAG GCC GTG GAG AAG GTC ACC GAG ACC CTG CTC AAG GGC GCC AAG GAG GTC GAG ACC AAG
K   A   V   E   K   V   T   E   T   L   L   K   G   A   K   E   V   E   T   K
423/141                                                             453/151
GAG CAG ATT GCG GCC ACC GCA GCG ATT TCG GCG GGT GAC CAG TCC ATC GGT GAC CTG ATC
E   Q   I   A   A   T   A   A   I   S   A   G   D   Q   S   I   G   D   L   I
483/161                                                             513/171
GCC GAG GCG ATG GAC AAG GTG GGC AAC GAG GGC GTC ATC ACC GTC GAG GAG TCC AAC ACC
A   E   A   M   D   K   V   G   N   E   G   V   I   T   V   E   E   S   N   T
543/181                                                             573/191
TTT GGG CTG CAG CTC GAG CTC ACC GAG GGT ATG CGG TTC GAC AAG GGC TAC ATC TCG GGG
F   G   L   Q   L   E   L   T   E   G   M   R   F   D   K   G   Y   I   S   G
603/201                                                             633/211
TAC TTC GTG ACC GAC CCG GAG CGT CAG GAG GCG GTC CTG GAG GAC CCC TAC ATC CTG CTG
Y   F   V   T   D   P   E   R   Q   E   A   V   L   E   D   P   Y   I   L   L
663/221                                                             693/231
GTC AGC TCC AAG GTG TCC ACT GTC AAG GAT CTG CTG CCG CTG CTC GAG AAG GTC ATC GGA
V   S   S   K   V   S   T   V   K   D   L   L   P   L   L   E   K   V   I   G
723/241                                                             753/251
GCC GGT AAG CCG CTG CTG ATC ATC GCC GAG GAC GTC GAG GGC GAG GCG CTG TCC ACC CTG
A   G   K   P   L   L   I   I   A   E   D   V   E   G   E   A   L   S   T   L
783/261                                                             813/271
GTC GTC AAC AAG ATC CGC GGC ACC TTC AAG TCG GTG GCG GTC AAG GCT CCC GGC TTC GGC
V   V   N   K   I   R   G   T   F   K   S   V   A   V   K   A   P   G   F   G
843/281                                                             873/291
GAC CGC CGC AAG GCG ATG CTG CAG GAT ATG GCC ATT CTC ACC GGT GGT CAG GTG ATC AGC
D   R   R   K   A   M   L   Q   D   M   A   I   L   T   G   G   Q   V   I   S
903/301                                                             933/311
GAA GAG GTC GGC CTG ACG CTG GAG AAC GCC GAC CTG TCG CTG CTA GGC AAG GCC CGC AAG
E   E   V   G   L   T   L   E   N   A   D   L   S   L   L   G   K   A   R   K
963/321                                                             993/331
GTC GTG GTC ACC AAG GAC GAG ACC ACC ATC GTC GAG GGC GCC GGT GAC ACC GAC GCC ATC
V   V   V   T   K   D   E   T   T   I   V   E   G   A   G   D   T   D   A   I
1023/341                                                            1053/351
GCC GGA CGA GTG GCC CAG ATC CGC CAG GAG ATC GAG AAC AGC GAC TCC GAC TAC GAC CGT
A   G   R   V   A   Q   I   R   Q   E   I   E   N   S   D   S   D   Y   D   R
1083/361                                                            1113/371
GAG AAG CTG CAG GAG CGG CTG GCC AAG CTG GCC GGT GGT GTC GCG GTG ATC AAG GCC GGT
E   K   L   Q   E   R   L   A   K   L   A   G   G   V   A   V   I   K   A   G
1143/381                                                            1173/391
GCC GCC ACC GAG GTC GAA CTC AAG GAG CGC AAG CAC CGC ATC GAG GAT GCG GTT CGC AAT
A   A   T   E   V   E   L   K   E   R   K   H   R   I   E   D   A   V   R   N
1203/401                                                            1233/411
GCC AAG GCC GCC GTC GAG GAG GGC ATC GTC GCC GGT GGG GGT GTG ACG CTG TTG CAA GCG
A   K   A   A   V   E   E   G   I   V   A   G   G   G   V   T   L   L   Q   A
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FIG. 4A

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1263/421                                                1293/431
GCC CCG ACC CTG GAC GAG CTG AAG CTC GAA GGC GAC GAG GCG ACC GGC GCC AAC ATC GTG
A   P   T   L   D   E   L   K   L   E   G   D   E   A   T   G   A   N   I   V
1323/441                                                1353/451
AAG GTG GCG CTG GAG GCC CCG CTG AAG CAG ATC GCC TTC AAC TCC GGG CTG GAG CCG GGC
K   V   A   L   E   A   P   L   K   Q   I   A   F   N   S   G   L   E   P   G
1383/461                                                1413/471
GTG GTG GCC GAG AAG GTG CGC AAC CTG CCG GCT GGC CAC GGA CTG AAC GCT CAG ACC GGT
V   V   A   E   K   V   R   N   L   P   A   G   H   G   L   N   A   Q   T   G
1443/481                                                1473/491
GTC TAC GAG GAT CTG CTC GCT GCC GGC GTT GCT GAC CCG GTC AAG GTG ACC CGT TCG GCG
V   Y   E   D   L   L   A   A   G   V   A   D   P   V   K   V   T   R   S   A
1503/501                                                1533/511
CTG CAG AAT GCG GCG TCC ATC GCG GGG CTG TTC CTG ACC ACC GAG GCC GTC GTT GCC GAC
L   Q   N   A   A   S   I   A   G   L   F   L   T   T   E   A   V   V   A   D
1563/521                                                1593/531
AAG CCG GAA AAG GAG AAG GCT TCC GTT CCC GGT GGC GGC GAC ATG GGT GGC ATG GAT TTC
K   P   E   K   E   K   A   S   V   P   G   G   G   D   M   G   G   M   D   F
1623/541                                                1653/551
cat atg cat gga gat aca cct aca ttg cat gaa tat atg tta gat ttg caa cca gag aca
H   M   H   G   D   T   P   T   L   H   E   Y   M   L   D   L   Q   P   E   T
1683/561                                                1713/571
act gat ctc tac tgt tat gag caa tta aat gac agc tca gag gag gag gat gaa ata gat
T   D   L   Y   C   Y   E   Q   L   N   D   S   S   E   E   E   D   E   I   D
1743/581                                                1773/591
ggt cca gct gga caa gca gaa ccg gac aga gcc cat tac aat att gta acc ttt tgt tgc
G   P   A   G   Q   A   E   P   D   R   A   H   Y   N   I   V   T   F   C   C
1803/601                                                1833/611
aag tgt gac tct acg ctt cgg ttg tgc gta caa agc aca cac gta gac att cgt act ttg
K   C   D   S   T   L   R   L   C   V   Q   S   T   H   V   D   I   R   T   L
1863/621                                                1893/631
gaa gac ctg tta atg ggc aca cta gga att gtg tgc ccc atc tgt tct cag aaa cca TAA
E   D   L   L   M   G   T   L   G   I   V   C   P   I   C   S   Q   K   P   *
```

FIG. 4B

```
1303/421                                                1333/431
GAG CAA TTA AAT GAC AGC TCA GAG GAG GAG GAT GAA ATA GAT GGT CCA GCT GGA CAA GCA
E   Q   L   N   D   S   S   E   E   E   D   E   I   D   G   P   A   G   Q   A
1363/441                                                1393/451
GAA CCG GAC AGA GCC CAT TAC AAT ATT GTA ACC TTT TGT TGC AAG TGT GAC TCT ACG CTT
E   P   D   R   A   H   Y   N   I   V   T   F   C   C   K   C   D   S   T   L
1423/461                                                1453/471
CGG TTG TGC GTA CAA AGC ACA CAC GTA GAC ATT CGT ACT TTG GAA GAC CTG TTA ATG GGC
R   L   C   V   Q   S   T   H   V   D   I   R   T   L   E   D   L   L   M   G
1483/481                                                1513/491
ACA CTA GGA ATT GTG TGC CCC ATC TGT TCT CAG AAA CCA TAG
T   L   G   I   V   C   P   I   C   S   Q   K   P   *
```

FIG. 5B

```
43/1                                                    73/11
ATG GCC CAA AGG GAA TGG GTC GAA AAA GAC TTC TAC CAG GAG CTG GGC GTC TCC TCT GAT
M   A   Q   R   E   W   V   E   K   D   F   Y   Q   E   L   G   V   S   S   D
103/21                                                  133/31
GCC AGT CCT GAA GAG ATC AAA CGT GCC TAT CGG AAG TTG GCG CGC GAC CTG CAT CCG GAC
A   S   P   E   E   I   K   R   A   Y   R   K   L   A   R   D   L   H   P   D
163/41                                                  193/51
GCG AAC CCG GGC AAC CCG GCC GCC GGC GAA CGG TTC AAG GCG GTT TCG GAG GCG CAT AAC
A   N   P   G   N   P   A   A   G   E   R   F   K   A   V   S   E   A   H   N
223/61                                                  253/71
GTG CTG TCG GAT CCG GCC AAG CGC AAG GAG TAC GAC GAA ACC CGC CGC CTG TTC GCC GGC
V   L   S   D   P   A   K   R   K   E   Y   D   E   T   R   R   L   F   A   G
283/81                                                  313/91
GGC GGG TTC GGC GGC CGT CGG TTC GAC AGC GGC TTT GGG GGC GGG TTC GGC GGT TTC GGG
G   G   F   G   G   R   R   F   D   S   G   F   G   G   G   F   G   G   F   G
343/101                                                 373/111
GTC GGT GGA GAC GGC GCC GAG TTC AAC CTC AAC GAC TTG TTC GAC GCC GCC AGC CGA ACC
V   G   G   D   G   A   E   F   N   L   N   D   L   F   D   A   A   S   R   T
403/121                                                 433/131
GGC GGT ACC ACC ATC GGT GAC TTG TTC GGT GGC TTG TTC GGA CGC GGT GGC AGC GCC CGT
G   G   T   T   I   G   D   L   F   G   G   L   F   G   R   G   G   S   A   R
463/141                                                 493/151
CCC AGC CGC CCG CGA CGC GGC AAC GAC CTG GAG ACC GAG ACC GAG TTG GAT TTC GTG GAG
P   S   R   P   R   R   G   N   D   L   E   T   E   T   E   L   D   F   V   E
523/161                                                 553/171
GCC GCC AAG GGC GTG GCG ATG CCG CTG CGA TTA ACC AGC CCG GCG CCG TGC ACC AAC TGC
A   A   K   G   V   A   M   P   L   R   L   T   S   P   A   P   C   T   N   C
583/181                                                 613/191
CAT GGC AGC GGG GCC CGG CCA GGC ACC AGC CCA AAG GTG TGT CCC ACT TGC AAC GGG TCG
H   G   S   G   A   R   P   G   T   S   P   K   V   C   P   T   C   N   G   S
643/201                                                 673/211
GGC GTG ATC AAC CGC AAT CAG GGC GCG TTC GGC TTC TCC GAG CCG TGC ACC GAC TGC CGA
G   V   I   N   R   N   Q   G   A   F   G   F   S   E   P   C   T   D   C   R
703/221                                                 733/231
GGT AGC GGC TCG ATC ATC GAG CAC CCC TGC GAG GAG TGC AAA GGC ACC GGC GTG ACC ACC
G   S   G   S   I   I   E   H   P   C   E   E   C   K   G   T   G   V   T   T
763/241                                                 793/251
CGC ACC CGA ACC ATC AAC GTG CGG ATC CCG CCC GGT GTC GAG GAT GGG CAG CGC ATC CGG
R   T   R   T   I   N   V   R   I   P   P   G   V   E   D   G   Q   R   I   R
823/261                                                 853/271
CTA GCC GGT CAG GGC GAG GCC GGG TTG CGC GGC GCT CCC TCG GGG GAT CTC TAC GTG ACG
L   A   G   Q   G   E   A   G   L   R   G   A   P   S   G   D   L   Y   V   T
883/281                                                 913/291
GTG CAT GTG CGG CCC GAC AAG ATC TTC GGC CGC GAC GGC GAC GAC CTC ACC GTC ACC GTT
V   H   V   R   P   D   K   I   F   G   R   D   G   D   D   L   T   V   T   V
943/301                                                 973/311
CCG GTC AGC TTC ACC GAA TTG GCT TTG GGC TCG ACG CTG TCG GTG CCT ACC CTG GAC GGC
P   V   S   F   T   E   L   A   L   G   S   T   L   S   V   P   T   L   D   G
1003/321                                                1033/331
ACG GTC GGG GTC CGG GTG CCC AAA GGC ACC GCT GAC GGC CGC ATT CTG CGT GTG CGC GGA
T   V   G   V   R   V   P   K   G   T   A   D   G   R   I   L   R   V   R   G
1063/341                                                1093/351
CGC GGT GTG CCC AAG CGC AGT GGG GGT AGC GGC GAC CTA CTT GTC ACC GTG AAG GTG GCC
R   G   V   P   K   R   S   G   G   S   G   D   L   L   V   T   V   K   V   A
1123/361                                                1153/371
GTG CCG CCC AAT TTG GCA GGC GCC GCT CAG GAA GCT CTG GAA GCC TAT GCG GCG GCG GAG
V   P   P   N   L   A   G   A   A   Q   E   A   L   E   A   Y   A   A   A   E
1183/381                                                1213/391
CGG TCC AGT GGT TTC AAC CCG CGG GCC GGA TGG GCA GGT AAT CGC ATG CAT GGA GAT ACA
R   S   S   G   F   N   P   R   A   G   W   A   G   N   R   M   H   G   D   T
1243/401                                                1273/411
CCT ACA TTG CAT GAA TAT ATG TTA GAT TTG CAA CCA GAG ACA ACT GAT CTC TAC TGT TAT
P   T   L   H   E   Y   M   L   D   L   Q   P   E   T   T   D   L   Y   C   Y
```

FIG. 5A

```
3/1                                                 33/11
ATG GGC AGC AGC CAT CAT CAT CAT CAT CAC AGC AGC GGC CTG GTG CCG CGC GGC AGC CAT
M   G   S   S   H   H   H   H   H   H   S   S   G   L   V   P   R   G   S   H
53/21                                               93/31
ATG gct agc atg ggc tcc atc ggc gca gca agc atg gaa ttt tgt ttt gat gta ttc aag
M   A   S   M   G   S   I   G   A   A   S   M   E   F   C   F   D   V   F   K
123/41                                              153/51
gag ctc aaa gtc cac cat gcc aat gag aac atc ttc tac tgc ccc att gcc atc atg tca
E   L   K   V   H   H   A   N   E   N   I   F   Y   C   P   I   A   I   M   S
183/61                                              213/71
gct cta gcc atg gta tac ctg ggt gca aaa gac agc acc agg aca cag ata aat aag gtt
A   L   A   M   V   Y   L   G   A   K   D   S   T   R   T   Q   I   N   K   V
243/81                                              273/91
gtt cgc ttt gat aaa ctt cca gga ttc gga gac agt att gaa gct cag tgt ggc aca tct
V   R   F   D   K   L   P   G   F   G   D   S   I   E   A   Q   C   G   T   S
303/101                                             333/111
gta aac gtt cac tct tca ctt aga gac atc ctc aac caa atc acc aaa cca aat gat gtt
V   N   V   H   S   S   L   R   D   I   L   N   Q   I   T   K   P   N   D   V
363/121                                             393/131
tat tcg ttc agc ctt gcc agt aga ctt tat gct gaa gag aga tac cca atc ctg cca gaa
Y   S   F   S   L   A   S   R   L   Y   A   E   E   R   Y   P   I   L   P   E
423/141                                             453/151
tac ttg cag tgt gtg aag gaa ctg tat aga gga ggc ttg gaa cct atc aac ttt caa aca
Y   L   Q   C   V   K   E   L   Y   R   G   G   L   E   P   I   N   F   Q   T
483/161                                             513/171
gct gca gat caa gcc aga gag ctc atc aat tcc tgg gta gaa agt cag aca aat gga att
A   A   D   Q   A   R   E   L   I   N   S   W   V   E   S   Q   T   N   G   I
543/181                                             573/191
atc aga aat gtc ctt cag cca agc tcc gtg gat tct caa act gca atg gtt ctg gtt aat
I   R   N   V   L   Q   P   S   S   V   D   S   Q   T   A   M   V   L   V   N
603/201                                             633/211
gcc att gtc ttc aaa gga ctg tgg gag aaa aca ttt aag gat gaa gac aca caa gca atg
A   I   V   F   K   G   L   W   E   K   T   F   K   D   E   D   T   Q   A   M
663/221                                             693/231
cct ttc aga gtg act gag caa gaa agc aaa cct gtg cag atg atg tac cag att ggt tta
P   F   R   V   T   E   Q   E   S   K   P   V   Q   M   M   Y   Q   I   G   L
723/241                                             753/251
ttt aga gtg gca tca atg gct tct gag aaa atg aag atc ctg gag ctt cca ttt gcc agt
F   R   V   A   S   M   A   S   E   K   M   K   I   L   E   L   P   F   A   S
783/261                                             813/271
ggg aca atg agc atg ttg gtg ctg ttg cct gat gaa gtc tca ggc ctt gag cag ctt gag
G   T   M   S   M   L   V   L   L   P   D   E   V   S   G   L   E   Q   L   E
843/281                                             873/291
agt ata atc aac ttt gaa aaa ctg act gaa tgg acc agt tct aat gtt atg gaa gag agg
S   I   I   N   F   E   K   L   T   E   W   T   S   S   N   V   M   E   E   R
903/301                                             933/311
aag atc aaa gtg tac tta cct cgc atg aag atg gag gaa aaa tac aac ctc aca tct gtc
K   I   K   V   Y   L   P   R   M   K   M   E   E   K   Y   N   L   T   S   V
963/321                                             993/331
tta atg gct atg ggc att act gac gtg ttt agc tct tca gcc aat ctg tct ggc atc tcc
L   M   A   M   G   I   T   D   V   F   S   S   S   A   N   L   S   G   I   S
1023/341                                            1053/351
tca gca gag agc ctg aag ata tct caa gct gtc cat gca gca cat gca gaa atc aat gaa
S   A   E   S   L   K   I   S   Q   A   V   H   A   A   H   A   E   I   N   E
1083/361                                            1113/371
gca ggc aga gag gtg gta ggg tca gca gag gct gga gtg gat gct gca agc gtc tct gaa
A   G   R   E   V   V   G   S   A   E   A   G   V   D   A   A   S   V   S   E
1143/381                                            1173/391
gaa ttt agg gct gac cat cca ttc ctc ttc tgt atc aag cac atc gca acc aac gcc gtt
E   F   R   A   D   H   P   F   L   F   C   I   K   H   I   A   T   N   A   V
1203/401
ctc ttc ttt ggc aga tgt gtt gga tcc taa
L   F   F   G   R   C   V   G   S   *
```

FIG. 6

```
3/1                                                                 33/11
atg gCC AGC AGC CAT CAT CAT CAT CAT CAC AGC AGC GGC CTG GTG CCG CGC GGC AGC cat
M   G   S   S   H   H   H   H   H   H   S   S   G   L   V   P   R   G   S   H
63/21                                                               93/31
atg GCC AAG ACA ATT GCG TAC GAC GAA GAG GCC CGT CGC GGC CTC GAG CGG GGC TTG AAC
M   A   K   T   I   A   Y   D   E   E   A   R   R   G   L   E   R   G   L   N
123/41                                                              153/51
GCC CTC GCC GAT GCG GTA AAG GTG ACA TTG GGC CCC AAG GGC CGC AAC GTC GTC CTG GAA
A   L   A   D   A   V   K   V   T   L   G   P   K   G   R   N   V   V   L   E
183/61                                                              213/71
AAG AAG TGG GGT GCC CCC ACG ATC ACC AAC GAT GGT GTG TCC ATC GCC AAG GAG ATC GAG
K   K   W   G   A   P   T   I   T   N   D   G   V   S   I   A   K   E   I   E
243/81                                                              273/91
CTG GAG GAT CCG TAC GAG aag atc ggc GCC GAG CTG GTC AAA GAG GTA GCC AAG AAG ACC
L   E   D   P   Y   E   K   I   G   A   E   L   V   K   E   V   A   K   K   T
303/101                                                             333/111
GAT GAC GTC GCC GGT GAC GGC ACC ACG ACG GCC ACC GTG CTG GCC CAG GCG TTG GTT CGC
D   D   V   A   G   D   G   T   T   T   A   T   V   L   A   Q   A   L   V   R
363/121                                                             393/131
gAg GGC CTG CGC AAC GTC GCG GCC GGC GCC AAC CCG CTC GGT CTC AAA CGC GGC ATC GAA
E   G   L   R   N   V   A   A   G   A   N   P   L   G   L   K   R   G   I   E
423/141                                                             453/151
AAG GCC GTG GAG AAG GTC ACC GAG ACC CTG CTC AAG GGC GCC AAG GAG GTC GAG ACC AAG
K   A   V   E   K   V   T   E   T   L   L   K   G   A   K   E   V   E   T   K
483/161                                                             513/171
GAG CAG ATT GCG GCC ACC GCA GCG ATT TCG GCG GGT GAC CAG TCC ATC GGT GAC CTG ATC
E   Q   I   A   A   T   A   A   I   S   A   G   D   Q   S   I   G   D   L   I
543/181                                                             573/191
GCC GAG GCG ATG GAC AAG GTG GGC AAC GAG GGC GTC ATC ACC GTC GAG GAG TCC AAC ACC
A   E   A   M   D   K   V   G   N   E   G   V   I   T   V   E   E   S   N   T
603/201                                                             633/211
TTT GGG CTG CAG CTC GAG CTC ACC GAG GGT ATG CGG TTC GAC AAG GGC TAC ATC TCG GGG
F   G   L   Q   L   E   L   T   E   G   M   R   F   D   K   G   Y   I   S   G
663/221                                                             693/231
TAC TTC GTG ACC GAC CCG GAG CGT CAG GAG GCG GTC CTG GAG GAC CCC TAC ATC CTG CTG
Y   F   V   T   D   P   E   R   Q   E   A   V   L   E   D   P   Y   I   L   L
723/241                                                             753/251
GTC AGC TCC AAG GTG TCC ACT GTC AAG GAT CTG CTG CCG CTG CTC GAG AAG GTC ATC GGA
V   S   S   K   V   S   T   V   K   D   L   L   P   L   L   E   K   V   I   G
783/261                                                             813/271
GCC GGT AAG CCG CTG CTG ATC ATC GCC GAG GAC GTC GAG GGC GAG GCG CTG TCC ACC CTG
A   G   K   P   L   L   I   I   A   E   D   V   E   G   E   A   L   S   T   L
843/281                                                             873/291
GTC GTC AAC AAG ATC CGC GGC ACC TTC AAG TCG GTG GCG GTC AAG GCT CCC GGC TTC GGC
V   V   N   K   I   R   G   T   F   K   S   V   A   V   K   A   P   G   F   G
903/301                                                             933/311
GAC CGC CGC AAG GCG ATG CTG CAG GAT ATG GCC ATT CTC ACC GGT GGT CAG GTG ATC AGC
D   R   R   K   A   M   L   Q   D   M   A   I   L   T   G   G   Q   V   I   S
963/321                                                             993/331
GAA GAG GTC GGC CTG ACG CTG GAG AAC GCC GAC CTG TCG CTG CTA GGC AAG GCC CGC AAG
E   E   V   G   L   T   L   E   N   A   D   L   S   L   L   G   K   A   R   K
1023/341                                                            1053/351
GTC GTG GTC ACC AAG GAC GAG ACC ACC ATC GTC GAG GGC GCC GGT GAC ACC GAC GCC ATC
V   V   V   T   K   D   E   T   T   I   V   E   G   A   G   D   T   D   A   I
1083/361                                                            1113/371
GCC CGA CGA GTG GCC CAG ATC CGC CAG GAG ATC GAG AAC AGC GAC TCC GAC TAC GAC CGT
A   G   R   V   A   Q   I   R   Q   E   I   E   N   S   D   S   D   Y   D   R
1143/381                                                            1173/391
GAG AAG CTG CAG GAG CGG CTG GCC AAG CTG GCC GGT GGT GTC GCG GTG ATC AAG GCC GGT
E   K   L   Q   E   R   L   A   K   L   A   G   G   V   A   V   I   K   A   G
1203/401                                                            1233/411
GCC GCC ACC GAG GTC GAA CTC AAG GAG CGC AAG CAC CGC ATC GAG GAT GCG GTT CGC AAT
A   A   T   E   V   E   L   K   E   R   K   H   R   I   E   D   A   V   R   N
```

FIG. 7A

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1263/421                                                    1293/431
GCC AAG GCC GCC GTC GAG GAG GGC ATC GTC GCC CGT GGG GGT GTG ACG CTG TTG CAA GCG
A   K   A   A   V   E   E   G   I   V   A   G   G   G   V   T   L   L   Q   A
1323/441                                                    1353/451
GCC CCG ACC CTG GAC GAG CTG AAG CTC GAA GGC GAC GAG GCG ACC GGC GCC AAC ATC GTG
A   P   T   L   D   E   L   K   L   E   G   D   E   A   T   G   A   N   I   V
1383/461                                                    1413/471
AAG GTG GCG CTG GAG GCC CCG CTG AAG CAG ATC GCC TTC AAC TCC GGG CTG GAG CCG GGC
K   V   A   L   E   A   P   L   K   Q   I   A   F   N   S   G   L   E   P   G
1443/481                                                    1473/491
GTG GTG GCC GAG AAG GTG CGC AAC CTG CCG GCT GGC CAC GGA CTG AAC GCT CAG ACC GGT
V   V   A   E   K   V   R   N   L   P   A   G   H   G   L   N   A   Q   T   G
1503/501                                                    1533/511
GTC TAC GAG GAT CTG CTC GCT GCC GGC GTT GCT GAC CCG GTC AAG GTG ACC CGT TCG GCC
V   Y   E   D   L   L   A   A   G   V   A   D   P   V   K   V   T   R   S   A
1563/521                                                    1593/531
CTG CAG AAT GCG GCG TCC ATC GCG GGG CTG TTC CTG ACC ACC GAG GCC GTC GTT GCC GAC
L   Q   N   A   A   S   I   A   G   L   F   L   T   T   E   A   V   V   A   D
1623/541                                                    1653/551
AAG CCG GAA AAG GAG AAG GCT TCC GTT CCC GGT GGC GGC GAC ATG GGT GGC ATG GAT TTC
K   P   E   K   E   K   A   S   V   P   G   G   G   D   M   G   G   M   D   F
1683/561                                                    1713/571
gct agc ATG ggc tcc atc ggc gca gca agc atg gaa ttt tgt ttt gat gta ttc aag gag
A   S   M   G   S   I   G   A   A   S   M   E   F   C   F   D   V   F   K   E
1743/581                                                    1773/591
ctc aaa gtc cac cat gcc aat gag aac atc ttc tac tgc ccc att gcc atc atg tca gct
L   K   V   H   H   A   N   E   N   I   F   Y   C   P   I   A   I   M   S   A
1803/601                                                    1833/611
cta gcc atg gta tac ctg ggt gca aaa gac agc acc agg aca cag ata aat aag gtt gtt
L   A   M   V   Y   L   G   A   K   D   S   T   R   T   Q   I   N   K   V   V
1863/621                                                    1893/631
cgc ttt gat aaa ctt cca gga ttc gga gac agt att gaa gct cag tgt ggc aca tct gta
R   F   D   K   L   P   G   F   G   D   S   I   E   A   Q   C   G   T   S   V
1923/641                                                    1953/651
aac gtt cac tct tca ctt aga gac atc ctc aac caa atc acc aaa cca aat gat gtt tat
N   V   H   S   S   L   R   D   I   L   N   Q   I   T   K   P   N   D   V   Y
1983/661                                                    2013/671
tcg ttc agc ctt gcc agt aga ctt tat gct gaa gag aga tac cca atc ctg cca gaa tac
S   F   S   L   A   S   R   L   Y   A   E   E   R   Y   P   I   L   P   E   Y
2043/681                                                    2073/691
ttg cag tgt gtg aag gaa ctg tat aga gga ggc ttg gaa cct atc aac ttt caa aca gct
L   Q   C   V   K   E   L   Y   R   G   G   L   E   P   I   N   F   Q   T   A
2103/701                                                    2133/711
gca gat caa gcc aga gag ctc atc aat tcc tgg gta gaa agt cag aca aat gga att atc
A   D   Q   A   R   E   L   I   N   S   W   V   E   S   Q   T   N   G   I   I
2163/721                                                    2193/731
aga aat gtc ctt cag cca agc tcc gtg gat tct caa act gca atg gtt ctg gtt aat gcc
R   N   V   L   Q   P   S   S   V   D   S   Q   T   A   M   V   L   V   N   A
2223/741                                                    2253/751
att gtc ttc aaa gga ctg tgg gag aaa aca ttt aag gat gaa gac aca caa gca atg cct
I   V   F   K   G   L   W   E   K   T   F   K   D   E   D   T   Q   A   M   P
2283/761                                                    2313/771
ttc aga gtg act gag caa gaa agc aaa cct gtg cag atg atg tac cag att ggt tta ttt
F   R   V   T   E   Q   E   S   K   P   V   Q   M   M   Y   Q   I   G   L   F
2343/781                                                    2373/791
aga gtg gca tca atg gct tct gag aaa atg aag atc ctg gag ctt cca ttt gcc agt ggg
R   V   A   S   M   A   S   E   K   M   K   I   L   E   L   P   F   A   S   G
2403/801                                                    2433/811
aca atg agc atg ttg gtg ctg ttg cct gat gaa gtc tca ggc ctt gag cag ctt gag agt
T   M   S   M   L   V   L   L   P   D   E   V   S   G   L   E   Q   L   E   S
2463/821                                                    2493/831
ata atc aac ttt gaa aaa ctg act gaa tgg acc agt tct aat gtt atg gaa gag agg aag
I   I   N   F   E   K   L   T   E   W   T   S   S   N   V   M   E   E   R   K
2523/841                                                    2553/851
atc aaa gtg tac tta cct cgc atg aag atg gag gaa aaa tac aac ctc aca tct gtc tta
I   K   V   Y   L   P   R   M   K   M   E   E   K   Y   N   L   T   S   V   L
2583/861                                                    2613/871
atg gct atg ggc att act gac gtg ttt agc tct tca gcc aat ctg tct ggc atc tcc tca
M   A   M   G   I   T   D   V   F   S   S   S   A   N   L   S   G   I   S   S
```

```
2643/881                                                2673/891
gca gag agc ctg aag ata tct caa gct gtc cat gca gca cat gca gaa atc aat gaa gca
A   E   S   L   K   I   S   Q   A   V   H   A   A   H   A   E   I   N   E   A
2703/901                                                2733/911
ggc aga gag gtg gta ggg tca gca gag gct gga gtg gat gct gca agc gtc tct gaa gaa
G   R   E   V   V   G   S   A   E   A   G   V   D   A   A   S   V   S   E   E
2763/921                                                2793/931
ttt agg gct gac cat cca ttc ctc ttc tgt atc aag cac atc gca acc aac gcc gtt ctc
F   R   A   D   H   P   F   L   F   C   I   K   H   I   A   T   N   A   V   L
2823/941
ttc ttt ggc aga tgt gtt gga tcc TAA
F   F   G   R   C   V   G   S   *
```

FIG. 7C

```
1/1                                                     31/11
atg tcc cct ata cta ggt tat tgg aaa att aag ggc ctt gtg caa ccc act cga ctt ctt
M   S   P   I   L   G   Y   W   K   I   K   G   L   V   Q   P   T   R   L   L
61/21                                                   91/31
ttg gaa tat ctt gaa gaa aaa tat gaa gag cat ttg tat gag cgc gat gaa ggt gat aaa
L   E   Y   L   E   E   K   Y   E   E   H   L   Y   E   R   D   E   G   D   K
121/41                                                  151/51
tgg cga aac aaa aag ttt gaa ttg ggt ttg gag ttt ccc aat ctt cct tat tat att gat
W   R   N   K   K   F   E   L   G   L   E   F   P   N   L   P   Y   Y   I   D
181/61                                                  211/71
ggt gat gtt aaa tta aca cag tct atg gcc atc ata cgt tat ata gct gac aag cac aac
G   D   V   K   L   T   Q   S   M   A   I   I   R   Y   I   A   D   K   H   N
241/81                                                  271/91
atg ttg ggt ggt tgt cca aaa gag cgt gca gag att tca atg ctt gaa gga gcg gtt ttg
M   L   G   G   C   P   K   E   R   A   E   I   S   M   L   E   G   A   V   L
301/101                                                 331/111
gat att aga tac ggt gtt tcg aga att gca tat agt aaa gac ttt gaa act ctc aaa gtt
D   I   R   Y   G   V   S   R   I   A   Y   S   K   D   F   E   T   L   K   V
361/121                                                 391/131
gat ttt ctt agc aag cta cct gaa atg ctg aaa atg ttc gaa gat cgt tta tgt cat aaa
D   F   L   S   K   L   P   E   M   L   K   M   F   E   D   R   L   C   H   K
421/141                                                 451/151
aca tat tta aat ggt gat cat gta acc cat cct gac ttc atg ttg tat gac gct ctt gat
T   Y   L   N   G   D   H   V   T   H   P   D   F   M   L   Y   D   A   L   D
481/161                                                 511/171
gtt gtt tta tac atg gac cca atg tgc ctg gat gcg ttc cca aaa tta gtt tgt ttt aaa
V   V   L   Y   M   D   P   M   C   L   D   A   F   P   K   L   V   C   F   K
541/181                                                 571/191
aaa cgt att gaa gct atc cca caa att gat aag tac ttg aaa tcc agc aag tat ata gca
K   R   I   E   A   I   P   Q   I   D   K   Y   L   K   S   S   K   Y   I   A
601/201                                                 631/211
tgg cct ttg cag ggc tgg caa gcc acg ttt ggt ggt ggc gac cat cct cca aaa tcg gat
W   P   L   Q   G   W   Q   A   T   F   G   G   G   D   H   P   P   K   S   D
661/221                                                 691/231
ctg gtt ccg cgt gga tcc cca gga att ccc ggg tcg aCT CGA GCA CCA CCA CCA CCA CCA
L   V   P   R   G   S   P   G   I   P   G   S   T   R   A   P   P   P   P   P
721/241
CTG AGA TCC GGC TGC TAA
L   R   S   G   C   *
```

FIG. 8

```
1/1                                                 31/11
atg tcc cct ata cta ggt tat tgg aaa att aag ggc ctt gtg caa ccc act cga ctt ctt
M   S   P   I   L   G   Y   W   K   I   K   G   L   V   Q   P   T   R   L   L
61/21                                               91/31
ttg gaa tat ctt gaa gaa aaa tat gaa gag cat ttg tat gag cgc gat gaa ggt gat aaa
L   E   Y   L   E   E   K   Y   E   E   H   L   Y   E   R   D   E   G   D   K
121/41                                              151/51
tgg cga aac aaa aag ttt gaa ttg ggt ttg gag ttt ccc aat ctt cct tat tat att gat
W   R   N   K   K   F   E   L   G   L   E   F   P   N   L   P   Y   Y   I   D
181/61                                              211/71
ggt gat gtt aaa tta aca cag tct atg gcc atc ata cgt tat ata gct gac aag cac aac
G   D   V   K   L   T   Q   S   M   A   I   I   R   Y   I   A   D   K   H   N
241/81                                              271/91
atg ttg ggt ggt tgt cca aaa gag cgt gca gag att tca atg ctt gaa gga gcg gtt ttg
M   L   G   G   C   P   K   E   R   A   E   I   S   M   L   E   G   A   V   L
301/101                                             331/111
gat att aga tac ggt gtt tcg aga att gca tat agt aaa gac ttt gaa act ctc aaa gtt
D   I   R   Y   G   V   S   R   I   A   Y   S   K   D   F   E   T   L   K   V
361/121                                             391/131
gat ttt ctt agc aag cta cct gaa atg ctg aaa atg ttc gaa gat cgt tta tgt cat aaa
D   F   L   S   K   L   P   E   M   L   K   M   F   E   D   R   L   C   H   K
421/141                                             451/151
aca tat tta aat ggt gat cat gta acc cat cct gac ttc atg ttg tat gac gct ctt gat
T   Y   L   N   G   D   H   V   T   H   P   D   F   M   L   Y   D   A   L   D
481/161                                             511/171
gtt gtt tta tac atg gac cca atg tgc ctg gat gcg ttc cca aaa tta gtt tgt ttt aaa
V   V   L   Y   M   D   P   M   C   L   D   A   F   P   K   L   V   C   F   K
541/181                                             571/191
aaa cgt att gaa gct atc cca caa att gat aag tac ttg aaa tcc agc aag tat ata gca
K   R   I   E   A   I   P   Q   I   D   K   Y   L   K   S   S   K   Y   I   A
601/201                                             631/211
tgg cct ttg cag ggc tgg caa gcc acg ttt ggt ggt ggc gac cat cct cca aaa tcg gat
W   P   L   Q   G   W   Q   A   T   F   G   G   G   D   H   P   P   K   S   D
661/221                                             691/231
ctg gtt ccg cgt gga tcc ATG CAT GGA GAT ACA CCT ACA TTG CAT GAA TAT ATG TTA GAT
L   V   P   R   G   S   M   H   G   D   T   P   T   L   H   E   Y   M   L   D
721/241                                             751/251
TTG CAA CCA GAG ACA ACT GAT CTC TAC TGT TAT GAG CAA TTA AAT GAC AGC TCA GAG GAG
L   Q   P   E   T   T   D   L   Y   C   Y   E   Q   L   N   D   S   S   E   E
781/261                                             811/271
GAG GAT GAA ATA GAT GGT CCA GCT GGA CAA GCA GAA CCG GAC AGA GCC CAT TAC AAT ATT
E   D   E   I   D   G   P   A   G   Q   A   E   P   D   R   A   H   Y   N   I
841/281                                             871/291
GTA ACC TTT TGT TGC AAG TGT GAC TCT ACG CTT CGG TTG TGC GTA CAA AGC ACA CAC GTA
V   T   F   C   C   K   C   D   S   T   L   R   L   C   V   Q   S   T   H   V
901/301                                             931/311
GAC ATT CGT ACT TTG GAA GAC CTG TTA ATG GGC ACA CTA GGA ATT GTG TGC CCC ATC TGT
D   I   R   T   L   E   D   L   L   M   G   T   L   G   I   V   C   P   I   C
961/321
TCT CAG AAA CCA TAA
S   Q   K   P   *
```

FIG. 9

```
3/1                                                 33/11
ATG GAT GGA GAT ACA CCT ACA TTG CAT GAA TAT ATG TTA GAT TTG CAA CCA GAG ACA ACT
M   D   G   D   T   P   T   L   H   E   Y   M   L   D   L   Q   P   E   T   T
63/21                                               93/31
GAT CTC TAC TGT TAT GAG CAA TTA AAT GAC AGC TCA GAG GAG GAG GAT GAA ATA GAT GGT
D   L   Y   C   Y   E   Q   L   N   D   S   S   E   E   E   D   E   I   D   G
123/41                                              153/51
CCA GCT GGA CAA GCA GAA CCG GAC AGA GCC CAT TAC AAT ATT GTA ACC TTT TGT TGC AAG
P   A   G   Q   A   E   P   D   R   A   H   Y   N   I   V   T   F   C   C   K
183/61                                              213/71
TGT GAC TCT ACG CTT CGG TTG TGC GTA CAA AGC ACA CAC GTA GAC ATT CGT ACT TTG GAA
C   D   S   T   L   R   L   C   V   Q   S   T   H   V   D   I   R   T   L   E
243/81                                              273/91
GAC CTG TTA ATG GGC ACA CTA GGA ATT GTG TGC CCC ATC TGT TCT CAG AAA CCA ACT AGT
D   L   L   M   G   T   L   G   I   V   C   P   I   C   S   Q   K   P   T   S
303/101                                             333/111
GGT GGC GGT GGC GGC GGA TCC CAC ATG GCC AAG ACA ATT GCG TAC GAC GAA GAG GCC CGT
G   G   G   G   G   G   S   H   M   A   K   T   I   A   Y   D   E   E   A   R
363/121                                             393/131
CGC GGC CTC GAG CGG GGC TTG AAC GCC CTC GCC GAT GCG GTA AAG GTG ACA TTG GGC CCC
R   G   L   E   R   G   L   N   A   L   A   D   A   V   K   V   T   L   G   P
423/141                                             453/151
AAG GGC CGC AAC GTC GTC CTG GAA AAG AAG TGG GGT GCC CCC ACG ATC ACC AAC GAT GGT
K   G   R   N   V   V   L   E   K   K   W   G   A   P   T   I   T   N   D   G
483/161                                             513/171
GTG TCC ATC GCC AAG GAG ATC GAG CTG GAG GAT CCG TAC GAG AAG ATC GGC GCC GAG CTG
V   S   I   A   K   E   I   E   L   E   D   P   Y   E   K   I   G   A   E   L
543/181                                             573/191
GTC AAA GAG GTA GCC AAG AAG ACC GAT GAC GTC GCC GGT GAC GGC ACC ACG ACG GCC ACC
V   K   E   V   A   K   K   T   D   D   V   A   G   D   G   T   T   T   A   T
603/201                                             633/211
GTG CTG GCC CAG GCG TTG GTT CGC GAG GGC CTG CGC AAC GTC GCG GCC GGC GCC AAC CCG
V   L   A   Q   A   L   V   R   E   G   L   R   N   V   A   A   G   A   N   P
663/221                                             693/231
CTC GGT CTC AAA CGC GGC ATC GAA AAG GCC GTG GAG AAG GTC ACC GAG ACC CTG CTC AAG
L   G   L   K   R   G   I   E   K   A   V   E   K   V   T   E   T   L   L   K
723/241                                             753/251
GGC GCC AAG GAG GTC GAG ACC AAG GAG CAG ATT GCG GCC ACC GCA GCG ATT TCG GCG GGT
G   A   K   E   V   E   T   K   E   Q   I   A   A   T   A   A   I   S   A   G
783/261                                             813/271
GAC CAG TCC ATC GGT GAC CTG ATC GCC GAG GCG ATG GAC AAG GTG GGC AAC GAG GGC GTC
D   Q   S   I   G   D   L   I   A   E   A   M   D   K   V   G   N   E   G   V
843/281                                             873/291
ATC ACC GTC GAG GAG TCC AAC ACC TTT GGG CTG CAG CTC GAG CTC ACC GAG GGT ATG CGG
I   T   V   E   E   S   N   T   F   G   L   Q   L   E   L   T   E   G   M   R
903/301                                             933/311
TTC GAC AAG GGC TAC ATC TCG GGG TAC TTC GTG ACC GAC CCG GAG CGT CAG GAG GCG GTC
F   D   K   G   Y   I   S   G   Y   F   V   T   D   P   E   R   Q   E   A   V
963/321                                             993/331
CTG GAG GAC CCC TAC ATC CTG CTG GTC AGC TCC AAG GTG TCC ACT GTC AAG GAT CTG CTG
L   E   D   P   Y   I   L   L   V   S   S   K   V   S   T   V   K   D   L   L
1023/341                                            1053/351
CCG CTG CTC GAG AAG GTC ATC GGA GCC GGT AAG CCG CTG CTG ATC ATC GCC GAG GAC GTC
P   L   L   E   K   V   I   G   A   G   K   P   L   L   I   I   A   E   D   V
1083/361                                            1113/371
GAG GGC GAG GCG CTG TCC ACC CTG GTC GTC AAC AAG ATC CGC GGC ACC TTC AAG TCG GTG
E   G   E   A   L   S   T   L   V   V   N   K   I   R   G   T   F   K   S   V
1143/381                                            1173/391
GCG GTC AAG GCT CCC GGC TTC GGC GAC CGC CGC AAG GCG ATG CTG CAG GAT ATG GCC ATT
A   V   K   A   P   G   F   G   D   R   R   K   A   M   L   Q   D   M   A   I
1203/401                                            1233/411
CTC ACC GGT GGT CAG GTG ATC AGC GAA GAG GTC GGC CTG ACG CTG GAG AAC GCC GAC CTG
L   T   G   G   Q   V   I   S   E   E   V   G   L   T   L   E   N   A   D   L
```

FIG. 10A

```
1263/421                                                1293/431
TCG CTG CTA GGC AAG GCC CGC AAG GTC GTG GTC ACC AAG GAC GAG ACC ACC ATC GTC GAG
S   L   L   G   K   A   R   K   V   V   V   T   K   D   E   T   T   I   V   E
1323/441                                                1353/451
GGC GCC GGT GAC ACC GAC GCC ATC GCC GGA CGA GTG GCC CAG ATC CGC CAG GAG ATC GAG
G   A   G   D   T   D   A   I   A   G   R   V   A   Q   I   R   Q   E   I   E
1383/461                                                1413/471
AAC AGC GAC TCC GAC TAC GAC CGT GAG AAG CTG CAG GAG CGG CTG GCC AAG CTG GCC GGT
N   S   D   S   D   Y   D   R   E   K   L   Q   E   R   L   A   K   L   A   G
1443/481                                                1473/491
GGT GTC GCG GTG ATC AAG GCC GGT GCC GCC ACC GAG GTC GAA CTC AAG GAG CGC AAG CAC
G   V   A   V   I   K   A   G   A   A   T   E   V   E   L   K   E   R   K   H
1503/501                                                1533/511
CGC ATC GAG GAT GCG GTT CGC AAT GCC AAG GCC GCC GTC GAG GAG GGC ATC GTC GCC GGT
R   I   E   D   A   V   R   N   A   K   A   A   V   E   E   G   I   V   A   G
1563/521                                                1593/531
GGG GGT GTG ACG CTG TTG CAA GCG GCC CCG ACC CTG GAC GAG CTG AAG CTC GAA GGC GAC
G   G   V   T   L   L   Q   A   A   P   T   L   D   E   L   K   L   E   G   D
1623/541                                                1653/551
GAG GCG ACC GGC GCC AAC ATC GTG AAG GTG GCG CTG GAG GCC CCG CTG AAG CAG ATC GCC
E   A   T   G   A   N   I   V   K   V   A   L   E   A   P   L   K   Q   I   A
1683/561                                                1713/571
TTC AAC TCC GGG CTG GAG CCG GGC GTG GTG GCC GAG AAG GTG CGC AAC CTG CCG GCT GGC
F   N   S   G   L   E   P   G   V   V   A   E   K   V   R   N   L   P   A   G
1743/581                                                1773/591
CAC GGA CTG AAC GCT CAG ACC GGT GTC TAC GAG GAT CTG CTC GCT GCC GGC GTT GCT GAC
H   G   L   N   A   Q   T   G   V   Y   E   D   L   L   A   A   G   V   A   D
1803/601                                                1833/611
CCG GTC AAG GTG ACC CGT TCG GCG CTG CAG AAT GCG GCG TCC ATC GCG GGG CTG TTC CTG
P   V   K   V   T   R   S   A   L   Q   N   A   A   S   I   A   G   L   F   L
1863/621                                                1893/631
ACC ACC GAG GCC GTC GTT GCC GAC AAG CCG GAA AAG GAG AAG GCT TCC GTT CCC GGT GGC
T   T   E   A   V   V   A   D   K   P   E   K   E   K   A   S   V   P   G   G
1923/641
GGC GAC ATG GGT GGC ATG GAT TTC TGA
G   D   M   G   G   M   D   F   *
```

FIG. 10B

```
3/1                                                                 33/11
ATG GCC AAG ACA ATT GCG TAC GAC GAA GAG GCC CGT CGC GGC CTC GAG CGG GGC TTG AAC
M   A   K   T   I   A   Y   D   E   E   A   R   R   G   L   E   R   G   L   N
63/21                                                               93/31
GCC CTC GCC GAT GCG GTA AAG GTG ACA TTG GGC CCC AAG GGC CGC AAC GTC GTC CTG GAA
A   L   A   D   A   V   K   V   T   L   G   P   K   G   R   N   V   V   L   E
123/41                                                              153/51
AAG AAG TGG GGT GCC CCC ACG ATC ACC AAC GAT GGT GTG TCC ATC GCC AAG GAG ATC GAG
K   K   W   G   A   P   T   I   T   N   D   G   V   S   I   A   K   E   I   E
183/61                                                              213/71
CTG GAG GAT CCG TAC GAG AAG ATC GGC GCC GAG CTG GTC AAA GAG GTA GCC AAG AAG ACC
L   E   D   P   Y   E   K   I   G   A   E   L   V   K   E   V   A   K   K   T
243/81                                                              273/91
GAT GAC GTC GCC GGT GAC GGC ACC ACG ACG GCC ACC GTG CTG GCC CAG GCG TTG GTT CGC
D   D   V   A   G   D   G   T   T   T   A   T   V   L   A   Q   A   L   V   R
303/101                                                             333/111
GAG GGC CTG CGC AAC GTC GCG GCC GGC GCC AAC CCG CTC GGT CTC AAA CGC GGC ATC GAA
E   G   L   R   N   V   A   A   G   A   N   P   L   G   L   K   R   G   I   E
363/121                                                             393/131
AAG GCC GTG GAG AAG GTC ACC GAG ACC CTG CTC AAG GGC GCC AAG GAG GTC GAG ACC AAG
K   A   V   E   K   V   T   E   T   L   L   K   G   A   K   E   V   E   T   K
423/141                                                             453/151
GAG CAG ATT GCG GCC ACC GCA GCG ATT TCG GCG GGT GAC CAG TCC ATC GGT GAC CTG ATC
E   Q   I   A   A   T   A   A   I   S   A   G   D   Q   S   I   G   D   L   I
483/161                                                             513/171
GCC GAG GCG ATG GAC AAG GTG GGC AAC GAG GGC GTC ATC ACC GTC GAG GAG TCC AAC ACC
A   E   A   M   D   K   V   G   N   E   G   V   I   T   V   E   E   S   N   T
543/181                                                             573/191
TTT GGG CTG CAG CTC GAG CTC ACC GAG GGT ATG CGG TTC GAC AAG GGC CAT ATG CAT GGA
F   G   L   Q   L   E   L   T   E   G   M   R   F   D   K   G   H   M   H   G
603/201                                                             633/211
GAT ACA CCT ACA TTG CAT GAA TAT ATG TTA GAT TTG CAA CCA GAG ACA ACT GAT CTC TAC
D   T   P   T   L   H   E   Y   M   L   D   L   Q   P   E   T   T   D   L   Y
663/221                                                             693/231
TGT TAT GAG CAA TTA AAT GAC AGC TCA GAG GAG GAG GAT GAA ATA GAT GGT CCA GCT GGA
C   Y   E   Q   L   N   D   S   S   E   E   E   D   E   I   D   G   P   A   G
723/241                                                             753/251
CAA GCA GAA CCG GAC AGA GCC CAT TAC AAT ATT GTA ACC TTT TGT TGC AAG TGT GAC TCT
Q   A   E   P   D   R   A   H   Y   N   I   V   T   F   C   C   K   C   D   S
783/261                                                             813/271
ACG CTT CGG TTG TGC GTA CAA AGC ACA CAC GTA GAC ATT CGT ACT TTG GAA GAC CTG TTA
T   L   R   L   C   V   Q   S   T   H   V   D   I   R   T   L   E   D   L   L
843/281                                                             873/291
ATG GGC ACA CTA GGA ATT GTG TGC CCC ATC TGT TCT CAG AAA CCA TAA
M   G   T   L   G   I   V   C   P   I   C   S   Q   K   P   *
```

FIG. 11

```
108/1
ATG GCG AAG GTG AAC ATC AAG CCA CTC GAG
M   A   K   V   N   I   K   P   L   E
168/21
GAG ACC ACG ACC GCG TCC GGT CTG GTC ATT
E   T   T   T   A   S   G   L   V   I
228/41
GGC ACC GTC GTT GCC GTC GGC CCT GGC CGG
G   T   V   V   A   V   G   P   G   R
288/61
CTG GAC GTT GCG GAG GGT GAC ACC GTC ATC
L   D   V   A   E   G   D   T   V   I
348/81
TAC AAC GGC GAG GAA TAC CTG ATC CTG TCG
Y   N   G   E   E   Y   L   I   L   S
408/101
ATG CAT GGA GAT ACA CCT ACA TTG CAT GAA
M   H   G   D   T   P   T   L   H   E
468/121
GAT CTC TAC TGT TAT GAG CAA TTA AAT GAC
D   L   Y   C   Y   E   Q   L   N   D
528/141
CCA GCT GGA CAA GCA GAA CCG GAC AGA GCC
P   A   G   Q   A   E   P   D   R   A
588/161
TGT GAC TCT ACG CTT CGG TTG TGC GTA CAA
C   D   S   T   L   R   L   C   V   Q
648/181
GAC CTG TTA ATG GGC ACA CTA GGA ATT GTG
D   L   L   M   G   T   L   G   I   V

138/11
GAC AAG ATT CTC GTG CAG GCC AAC GAG GCC
D   K   I   L   V   Q   A   N   E   A
198/31
CCT GAC ACC GCC AAG GAG AAG CCG CAG GAG
P   D   T   A   K   E   K   P   Q   E
258/51
TGG GAC GAG GAC GGC GAG AAG CGG ATC CCG
W   D   E   D   G   E   K   R   I   P
318/71
TAC AGC AAG TAC GGC GGC ACC GAG ATC AAG
Y   S   K   Y   G   G   T   E   I   K
378/91
GCA CGC GAC GTG CTG GCC GTC GTT TCC AAG
A   R   D   V   L   A   V   V   S   K
438/111
TAT ATG TTA GAT TTG CAA CCA GAG ACA ACT
Y   M   L   D   L   Q   P   E   T   T
498/131
AGC TCA GAG GAG GAG GAT GAA ATA GAT GGT
S   S   E   E   E   D   E   I   D   G
558/151
CAT TAC AAT ATT GTA ACC TTT TGT TGC AAG
H   Y   N   I   V   T   F   C   C   K
618/171
AGC ACA CAC GTA GAC ATT CGT ACT TTG GAA
S   T   H   V   D   I   R   T   L   E
678/191
TGC CCC ATC TGT TCT CAG AAA CCA TAG
C   P   I   C   S   Q   K   P   *
```

FIG. 12

```
3/1                                                                 33/11
atg gat gga gat aca cct aca ttg cat gaa tat atg tta gat ttg caa cca gag aca act
M   D   G   D   T   P   T   L   H   E   Y   M   L   D   L   Q   P   E   T   T
63/21                                                               93/31
gat ctc tac tgt tat gag caa tta aat gac agc tca gag gag gag gat gaa ata gat ggt
D   L   Y   C   Y   E   Q   L   N   D   S   S   E   E   E   D   E   I   D   G
123/41                                                              153/51
cca gct gga caa gca gaa ccg gac aga gcc cat tac aat att gta acc ttt tgt tgc aag
P   A   G   Q   A   E   P   D   R   A   H   Y   N   I   V   T   F   C   C   K
183/61                                                              213/71
tgt gac tct acg ctt cgg ttg tgc gta caa agc aca cac gta gac att cgt act ttg gaa
C   D   S   T   L   R   L   C   V   Q   S   T   H   V   D   I   R   T   L   E
243/81                                                              273/91
gac ctg tta atg ggc aca cta gga att gtg tgc ccc atc tgt tct cag aaa cca gcc atg
D   L   L   M   G   T   L   G   I   V   C   P   I   C   S   Q   K   P   A   M
303/101                                                             333/111
gCT CGT GCG GTC GGG ATC GAC CTC GGG ACC ACC AAC TCC GTC GTC TCG GTT CTG GAA GGT
A   R   A   V   G   I   D   L   G   T   T   N   S   V   V   S   V   L   E   G
363/121                                                             393/131
GGC GAC CCG GTC GTC GTC GCC AAC TCC GAG GGC TCC AGG ACC ACC CCG TCA ATT GTC GCG
G   D   P   V   V   V   A   N   S   E   G   S   R   T   T   P   S   I   V   A
423/141                                                             453/151
TTC GCC CGC AAC GGT GAG GTG CTG GTC GGC CAG CCC GCC AAG AAC CAG GCG GTG ACC AAC
F   A   R   N   G   E   V   L   V   G   Q   P   A   K   N   Q   A   V   T   N
483/161                                                             513/171
GTC GAT CGC ACC GTG CGC TCG GTC AAG CGA CAC ATG GGC AGC GAC TGG TCC ATA GAG ATT
V   D   R   T   V   R   S   V   K   R   H   M   G   S   D   W   S   I   E   I
543/181                                                             573/191
GAC GGC AAG AAA TAC ACC GCG CCG GAG ATC AGC GCC CGC ATT CTG ATG AAG CTG AAG CGC
D   G   K   K   Y   T   A   P   E   I   S   A   R   I   L   M   K   L   K   R
603/201                                                             633/211
GAC GCC GAG GCC TAC CTC GGT GAG GAC ATT ACC GAC GCG GTT ATC ACG ACG CCC GCC TAC
D   A   E   A   Y   L   G   E   D   I   T   D   A   V   I   T   T   P   A   Y
663/221                                                             693/231
TTC AAT GAC GCC CAG CGT CAG GCC ACC AAG GAC GCC GGC CAG ATC GCC GGC CTC AAC GTG
F   N   D   A   Q   R   Q   A   T   K   D   A   G   Q   I   A   G   L   N   V
723/241                                                             753/251
CTG CGG ATC GTC AAC GAG CCG ACC GCG GCC GCG ctg gcc TAC GGC CTC GAC AAG GGC GAG
L   R   I   V   N   E   P   T   A   A   A   L   A   Y   G   L   D   K   G   E
783/261                                                             813/271
AAG GAG CAG CGA ATC CTG GTC TTC GAC TTG GGT GGT GGC ACT TTC GAC GTT TCC CTG CTG
K   E   Q   R   I   L   V   F   D   L   G   G   G   T   F   D   V   S   L   L
843/281                                                             873/291
GAG ATC GGC GAG GGT GTG GTT GAG GTC CGT GCC ACT TCG GGT GAC AAC CAC CTC GGC GGC
E   I   G   E   G   V   V   E   V   R   A   T   S   G   D   N   H   L   G   G
903/301                                                             933/311
GAC GAC TGG GAC CAG CGG GTC GTC GAT TGG CTG GTG GAC AAG TTC AAG GGC ACC AGC GGC
D   D   W   D   Q   R   V   V   D   W   L   V   D   K   F   K   G   T   S   G
963/321                                                             993/331
ATC GAT CTG ACC AAG GAC AAG ATG GCG ATG CAG CGG CTG CGG GAA GCC GCC GAG AAG GCA
I   D   L   T   K   D   K   M   A   M   Q   R   L   R   E   A   A   E   K   A
1023/341                                                            1053/351
AAG ATC GAG CTG AGT TCG AGT CAG TCC ACC TCG ATC AAC CTG CCC TAC ATC ACC GTC GAC
K   I   E   L   S   S   S   Q   S   T   S   I   N   L   P   Y   I   T   V   D
1083/361                                                            1113/371
GCC GAC AAG AAC CCC TTG TTC TTA GAC GAG CAG CTG ACC CGC GCG GAG TTC CAA CGG ATC
A   D   K   N   P   L   F   L   D   E   Q   L   T   R   A   E   F   Q   R   I
1143/381                                                            1173/391
ACT CAG GAC CTG CTG GAC CGC ACT CGC AAG CCG TTC CAG TCG GTG ATC GCT GAC ACC GGC
T   Q   D   L   L   D   R   T   R   K   P   F   Q   S   V   I   A   D   T   G
1203/401                                                            1233/411
ATT TCG GTG TCG GAG ATC GAT CAC GTT GTG CTC GTG GGT GGT TCG ACC CGG ATG CCC GCG
I   S   V   S   E   I   D   H   V   V   L   V   G   G   S   T   R   M   P   A
```

FIG. 13A

```
1263/421                                                1293/431
GTG ACC GAT CTG GTC AAG GAA CTC ACC GGC GGC AAG GAA CCC AAC AAG GGC GTC AAC CCC
V   T   D   L   V   K   E   L   T   G   G   K   E   P   N   K   G   V   N   P
1323/441                                                1353/451
GAT GAG GTT GTC GCG GTG GGA GCC GCT CTG CAG GCC GGC GTC CTC AAG GGC GAG GTG AAA
D   E   V   V   A   V   G   A   A   L   Q   A   G   V   L   K   G   E   V   K
1383/461                                                1413/471
GAC GTT CTG CTG CTT GAT GTT ACC CCG CTG AGC CTG GGT ATC GAG ACC AAG GGC GGG GTG
D   V   L   L   L   D   V   T   P   L   S   L   G   I   E   T   K   G   G   V
1443/481                                                1473/491
ATG ACC AGG CTC ATC GAG CGC AAC ACC ACG ATC CCC ACC AAG CGG TCG GAG ACT TTC ACC
M   T   R   L   I   E   R   N   T   T   I   P   T   K   R   S   E   T   F   T
1503/501                                                1533/511
ACC GCC GAC GAC AAC CAA CCG TCG GTG CAG ATC CAG GTC TAT CAG GGG GAG CGT GAG ATC
T   A   D   D   N   Q   P   S   V   Q   I   Q   V   Y   Q   G   E   R   E   I
1563/521                                                1593/531
GCC GCG CAC AAC AAG TTG CTC GGG TCC TTC GAG CTG ACC GGC ATC CCG CCG GCG CCG CGG
A   A   H   N   K   L   L   G   S   F   E   L   T   G   I   P   P   A   P   R
1623/541                                                1653/551
GGG ATT CCG CAG ATC GAG GTC ACT TTC GAC ATC GAC GCC AAC GGC ATT GTG CAC GTC ACC
G   I   P   Q   I   E   V   T   F   D   I   D   A   N   G   I   V   H   V   T
1683/561                                                1713/571
GCC AAG GAC AAG GGC ACC GGC AAG GAG AAC ACG ATC CGA ATC CAG GAA GGC TCG GGC CTG
A   K   D   K   G   T   G   K   E   N   T   I   R   I   Q   E   G   S   G   L
1743/581                                                1773/591
TCC AAG GAA GAC ATT GAC CGC ATG ATC AAG GAC GCC GAA GCG CAC GCC GAG GAG GAT CGC
S   K   E   D   I   D   R   M   I   K   D   A   E   A   H   A   E   E   D   R
1803/601                                                1833/611
AAG CGT CGC GAG GAG GCC GAT GTT CGT AAT CAA GCC GAG ACA TTG GTC TAC CAG ACG GAG
K   R   R   E   E   A   D   V   R   N   Q   A   E   T   L   V   Y   Q   T   E
1863/621                                                1893/631
AAG TTC GTC AAA GAA CAG CGT GAG GCC GAG GGT GGT TCG AAG GTA CCT GAA GAC ACG CTG
K   F   V   K   E   Q   R   E   A   E   G   G   S   K   V   P   E   D   T   L
1923/641                                                1953/651
AAC AAG GTT GAT GCC GCG GTG GCG GAA GCg AAG GCG GCA CTT GGC GGA TCG GAT ATT TCG
N   K   V   D   A   A   V   A   E   A   K   A   A   L   G   G   S   D   I   S
1983/661                                                2013/671
GCC ATC AAG TCG GCG ATG GAG AAG CTG GGC CAG GAG TCG CAG GCT CTG GGG CAA GCG ATC
A   I   K   S   A   M   E   K   L   G   Q   E   S   Q   A   L   G   Q   A   I
2043/681                                                2073/691
TAC GAA GCA GCT CAG GCT GCG TCA CAG GCC ACT GGC GCT GCC CAC CCC GGC GGC GAG CCG
Y   E   A   A   Q   A   A   S   Q   A   T   G   A   A   H   P   G   G   E   P
2103/701                                                2133/711
GGC GGT GCC CAC CCC GGC TCG GCT GAG CTA GCA TGA
G   G   A   H   P   G   S   A   E   L   A   *
```

FIG. 13B

```
3/1                                                                 33/11
atg gat gga gat aca cct aca ttg cat gaa tat atg tta gat ttg caa cca gag aca act
M   D   G   D   T   P   T   L   H   E   Y   M   L   D   L   Q   P   E   T   T
63/21                                                               93/31
gat ctc tac tgt tat gag caa tta aat gac agc tca gag gag gag gat gaa ata gat ggt
D   L   Y   C   Y   E   Q   L   N   D   S   S   E   E   E   D   E   I   D   G
123/41                                                              153/51
cca gct gga caa gca gaa ccg gac aga gcc cat tac aat att gta acc ttt tgt tgc aag
P   A   G   Q   A   E   P   D   R   A   H   Y   N   I   V   T   F   C   C   K
183/61                                                              213/71
tgt gac tct acg ctt cgg ttg tgc gta caa agc aca cac gta gac att cgt act ttg gaa
C   D   S   T   L   R   L   C   V   Q   S   T   H   V   D   I   R   T   L   E
243/81                                                              273/91
gac ctg tta atg ggc aca cta gga att gtg tgc ccc atc tgt tct cag aaa cca gcc atg
D   L   L   M   G   T   L   G   I   V   C   P   I   C   S   Q   K   P   A   M
303/101                                                             333/111
gCT CGT GCG GTC GGG ATC GAC CTC GGG ACC ACC AAC TCC GTC GTC TCG GTT CTG GAA GGT
A   R   A   V   G   I   D   L   G   T   T   N   S   V   V   S   V   L   E   G
363/121                                                             393/131
GGC GAC CCG GTC GTC GTC GCC AAC TCC GAG GGC TCC AGG ACC ACC CCG TCA ATT GTC GCG
G   D   P   V   V   V   A   N   S   E   G   S   R   T   T   P   S   I   V   A
423/141                                                             453/151
TTC GCC CGC AAC GGT GAG GTG CTG GTC GGC CAG CCC GCC AAG AAC CAG GCG GTG ACC AAC
F   A   R   N   G   E   V   L   V   G   Q   P   A   K   N   Q   A   V   T   N
483/161                                                             513/171
GTC GAT CGC ACC GTG CGC TCG GTC AAG CGA CAC ATG GGC AGC GAC TGG TCC ATA GAG ATT
V   D   R   T   V   R   S   V   K   R   H   M   G   S   D   W   S   I   E   I
543/181                                                             573/191
GAC GGC AAG AAA TAC ACC GCG CCG GAG ATC AGC GCC CGC ATT CTG ATG AAG CTG AAG CGC
D   G   K   K   Y   T   A   P   E   I   S   A   R   I   L   M   K   L   K   R
603/201                                                             633/211
GAC GCC GAG GCC TAC CTC GGT GAG GAC ATT ACC GAC GCG GTT ATC ACG ACG CCC GCC TAC
D   A   E   A   Y   L   G   E   D   I   T   D   A   V   I   T   T   P   A   Y
663/221                                                             693/231
TTC AAT GAC GCC CAG CGT CAG GCC ACC AAG GAC GCC GGC CAG ATC GCC GGC CTC AAC GTG
F   N   D   A   Q   R   Q   A   T   K   D   A   G   Q   I   A   G   L   N   V
723/241                                                             753/251
CTG CGG ATC GTC AAC GAG CCG ACC GCG GCC GCG ctg gcc TAC GGC CTC GAC AAG GGC GAG
L   R   I   V   N   E   P   T   A   A   A   L   A   Y   G   L   D   K   G   E
783/261                                                             813/271
AAG GAG CAG CGA ATC CTG GTC TTC GAC TTG GGT GGT GGC ACT TTC GAC GTT TCC CTG CTG
K   E   Q   R   I   L   V   F   D   L   G   G   G   T   F   D   V   S   L   L
843/281                                                             873/291
GAG ATC GGC GAG GGT GTG GTT GAG GTC CGT GCC ACT TCG GGT GAC AAC CAC CTC GGC GGC
E   I   G   E   G   V   V   E   V   R   A   T   S   G   D   N   H   L   G   G
903/301                                                             933/311
GAC GAC TGG GAC CAG CGG GTC GTC GAT TGG CTG GTG GAC AAG TTC AAG GGC ACC AGC GGC
D   D   W   D   Q   R   V   V   D   W   L   V   D   K   F   K   G   T   S   G
963/321                                                             993/331
ATC GAT CTG ACC AAG GAC AAG ATG GCG ATG CAG CGG CTG CGG GAA GCC GCC GAG AAG GCA
I   D   L   T   K   D   K   M   A   M   Q   R   L   R   E   A   A   E   K   A
1023/341                                                            1053/351
AAG ATC GAG CTG AGT TCG AGT CAG TCC ACC TCG ATC AAC CTG CCC TAC ATC ACC GTC GAC
K   I   E   L   S   S   S   Q   S   T   S   I   N   L   P   Y   I   T   V   D
1083/361                                                            1113/371
GCC GAC AAG AAC CCG TTG TTC TTA GAC GAG CAG CTG ACC CGC GCG GAG TTC CAA CGG ATC
A   D   K   N   P   L   F   L   D   E   Q   L   T   R   A   E   F   Q   R   I
1143/381                                                            1173/391
ACT CAG GAC CTG CTG GAC CGC ACT CGC AAG CCG TTC CAG TCG GTG ATC GCT GAC ACC GGC
T   Q   D   L   L   D   R   T   R   K   P   F   Q   S   V   I   A   D   T   G
1203/401                                                            1233/411
ATT TCG GTG TCG GAG ATC GAT CAC GTT GTG CTC GTG GGT GGT TCG ACC CGG ATC CCC GCG
I   S   V   S   E   I   D   H   V   V   L   V   G   G   S   T   R   M   P   A
```

FIG. 14A

```
1263/421                                                    1293/431
GTG ACC GAT CTG GTC AAG GAA CTC ACC GGC GGC AAG GAA CCC AAC AAG GGC GTC AAC CCC
V   T   D   L   V   K   E   L   T   G   G   K   E   P   N   K   G   V   N   P
1323/441                                                    1353/451
GAT GAG GTT GTC GCG GTG GGA GCC GCT CTG CAG GCC GGC GTC CTC AAG GGC GAG GTG AAA
D   E   V   V   A   V   G   A   A   L   Q   A   G   V   L   K   G   E   V   K
1383/461                                                    1413/471
GAC GTT CTG CTG CTT GAT GTT ACC CCG CTG AGC CTG GGT ATC GAG ACC AAG GGC GGG GTG
D   V   L   L   L   D   V   T   P   L   S   L   G   I   E   T   K   G   G   V
1443/481                                                    1473/491
ATG ACC AGG CTC ATC GAG CGC AAC ACC ACG ATC CCC ACC AAG CGG TCG GAG ACT TTC ACC
M   T   R   L   I   E   R   N   T   T   I   P   T   K   R   S   E   T   F   T
1503/501                                                    1533/511
ACC GCC GAC GAC AAC CAA CCG TCG GTG CAG ATC CAG GTC TAT CAG GGG GAG CGT GAG ATC
T   A   D   D   N   Q   P   S   V   Q   I   Q   V   Y   Q   G   E   R   E   I
1563/521                                                    1593/531
GCC GCG CAC AAC AAG TTG CTC GGG TCC TTC GAG CTG ACC GGC ATC CCG CCG GCG CCG CGG
A   A   H   N   K   L   L   G   S   F   E   L   T   G   I   P   P   A   P   R
1623/541                                                    1653/551
GGG ATT CCG CAG ATC GAG GTC ACT TTC GAC ATC GAC GCC AAC GGC ATT GTG CAC GTC ACC
G   I   P   Q   I   E   V   T   F   D   I   D   A   N   G   I   V   H   V   T
1683/561                                                    1713/571
GCC AAG GAC AAG GGC ACC GGC AAG GAG AAC ACG ATC CGA ATC CAG GAA GGC TCG GGC CTG
A   K   D   K   G   T   G   K   E   N   T   I   R   I   Q   E   G   S   G   L
1743/581                                                    1773/591
TCC AAG GAA GAC ATT GAC CGC ATG ATC AAG GAC GCC GAA GCG CAC GCC GAG GAG GAT CGC
S   K   E   D   I   D   R   M   I   K   D   A   E   A   H   A   E   E   D   R
1803/601                                                    1833/611
AAG CGT CGC GAG GAG GCC GAT GTT CGT AAT CAA GCC GAG ACA TTG GTC TAC CAG ACG GAG
K   R   R   E   E   A   D   V   R   N   Q   A   E   T   L   V   Y   Q   T   E
1863/621                                                    1893/631
AAG TTC GTC AAA GAA CAG CGT GAG GCC GAG GGT GGT TCG AAg gta ccT GAA GAC ACG CTG
K   F   V   K   E   Q   R   E   A   E   G   G   S   K   V   P   E   D   T   L
1923/641                                                    1953/651
AAC AAG GTT GAT GCC GCG GTG GCG GAA GCg AAG GCG GCA CTT GGC GGA TCG GAT ATT TCG
N   K   V   D   A   A   V   A   E   A   K   A   A   L   G   G   S   D   I   S
1983/661                                                    2013/671
GCC ATC AAG TCG GCG ATG GAG AAG CTG GGC CAG GAG TCG CAG GCT CTG GGG CAA GCG ATC
A   I   K   S   A   M   E   K   L   G   Q   E   S   Q   A   L   G   Q   A   I
2043/681                                                    2073/691
TAC GAA GCA GCT CAG GCT GCG TCA CAG GCC ACT GGC GCT GCC CAC CCC GGC GGC GAG CCG
Y   E   A   A   Q   A   A   S   Q   A   T   G   A   A   H   P   G   G   E   P
2103/701                                                    2133/711
GGC GGT GCC CAC CCC GGC TCG GCT GAT GAC GTT GTG GAC GCG GAG GTG GTC GAC GAC GGC
G   G   A   H   P   G   S   A   D   D   V   V   D   A   E   V   V   D   D   G
2163/721
CGG GAG GCC AAG TGA
R   E   A   K   *
```

FIG. 14B

```
3/1                                                                 33/11
atg gCA AAA GAA ATT AAA TTT TCA TCA GAT GCC CGT TCA GCT ATG GTC CGT GGT GTC GAT
M   A   K   E   I   K   F   S   S   D   A   R   S   A   M   V   R   G   V   D
63/21                                                               93/31
ATC CTT GCA GAT ACT GTT AAA GTA ACT TTG GGA CCA AAA GGT CGC AAT GTC GTT CTT GAA
I   L   A   D   T   V   K   V   T   L   G   P   K   G   R   N   V   V   L   E
123/41                                                              153/51
AAG TCA TTC GGT TCA CCC TTG ATT ACC AAT GAC GGT GTG ACT ATT GCC AAA GAA ATT GAA
K   S   F   G   S   P   L   I   T   N   D   G   V   T   I   A   K   E   I   E
183/61                                                              213/71
TTA GAA GAC CAT TTT GAA AAT ATG GGT GCC AAA TTG GTA TCA GAA GTA GCT TCA AAA ACC
L   E   D   H   F   E   N   M   G   A   K   L   V   S   E   V   A   S   K   T
243/81                                                              273/91
AAT GAT ATC GCA GGT GAT GGA ACT ACA ACT GCA ACT GTT TTG ACC CAA GCA ATC GTC CGT
N   D   I   A   G   D   G   T   T   T   A   T   V   L   T   Q   A   I   V   R
303/101                                                             333/111
GAA GGA ATC AAA AAC GTC ACA GCA GGT GCA AAT CCA ATC GGT ATT CGT CGT GGG ATT GAA
E   G   I   K   N   V   T   A   G   A   N   P   I   G   I   R   R   G   I   E
363/121                                                             393/131
ACA GCA GTT GCC GCA GCA GTT GAA GCT TTG AAA AAC AAC GTC ATC CCT GTT GCC AAT AAA
T   A   V   A   A   A   V   E   A   L   K   N   N   V   I   P   V   A   N   K
423/141                                                             453/151
GAA GCT ATC GCT CAA GTT GCA GCC GTA TCT TCT CGT TCT GAA AAA GTT GGT GAG TAC ATC
E   A   I   A   Q   V   A   A   V   S   S   R   S   E   K   V   G   E   Y   I
483/161                                                             513/171
TCT GAA GCA ATG GAA AAA GTT GGC AAA GAC GGT GTC ATC ACC ATC GAA GAG TCA CGT GGT
S   E   A   M   E   K   V   G   K   D   G   V   I   T   I   E   E   S   R   G
543/181                                                             573/191
ATG GAA ACA GAG CTT GAA GTC GTA GAA GGA ATG CAG TTT GAC CGT GGT TAC CTT TCA CAG
M   E   T   E   L   E   V   V   E   G   M   Q   F   D   R   G   Y   L   S   Q
603/201                                                             633/211
TAC ATG GTG ACA GAT AGC GAA AAA ATG GTG GCT GAC CTT GAA AAT CCG TAC ATT TTG ATT
Y   M   V   T   D   S   E   K   M   V   A   D   L   E   N   P   Y   I   L   I
663/221                                                             693/231
ACA GAC AAG AAA ATT TCC AAT ATC CAA GAA ATC TTG CCA CTT TTG GAA AGC ATT CTC CAA
T   D   K   K   I   S   N   I   Q   E   I   L   P   L   L   E   S   I   L   Q
723/241                                                             753/251
AGC AAT CGT CCA CTC TTG ATT ATT GCG GAT GAT GTG GAT GGT GAG GCT CTT CCA ACT CTT
S   N   R   P   L   L   I   I   A   D   D   V   D   G   E   A   L   P   T   L
783/261                                                             813/271
GTT TTG AAC AAG ATT CGT GGA ACC TTC AAC GTA GTA GCA GTC AAG GCA CCT GGT TTT GGT
V   L   N   K   I   R   G   T   F   N   V   V   A   V   K   A   P   G   F   G
843/281                                                             873/291
GAC CGT CGC AAA GCC ATG CTT GAA GAT ATC GCC ATC TTA ACA GGC GGA ACA GTT ATC ACA
D   R   R   K   A   M   L   E   D   I   A   I   L   T   G   G   T   V   I   T
903/301                                                             933/311
GAA GAC CTT GGT CTT GAG TTG AAA GAT GCG ACA ATT GAA GCT CTT GGT CAA GCA GCG AGA
E   D   L   G   L   E   L   K   D   A   T   I   E   A   L   G   Q   A   A   R
963/321                                                             993/331
GTG ACC GTG GAC AAA GAT AGC ACG GTT ATT GTA GAA GGT GCA GGA AAT CCT GAA GCG ATT
V   T   V   D   K   D   S   T   V   I   V   E   G   A   G   N   P   E   A   I
1023/341                                                            1053/351
TCT CAC CGT GTT GCG GTT ATC AAG TCT CAA ATC GAA ACT ACA ACT TCT GAA TTT GAC CGT
S   H   R   V   A   V   I   K   S   Q   I   E   T   T   T   S   E   F   D   R
1083/361                                                            1113/371
GAA AAA TTG CAA GAA CGC TTG GCC AAA TTG TCA GGT GGT GTA GCG GTT ATT AAG GTC GGA
E   K   L   Q   E   R   L   A   K   L   S   G   G   V   A   V   I   K   V   G
1143/381                                                            1173/391
GCC GCA ACT GAA ACT GAG TTG AAA GAA ATG AAA CTC CGC ATT GAA GAT GCC CTC AAC GCT
A   A   T   E   T   E   L   K   E   M   K   L   R   I   E   D   A   L   N   A
1203/401                                                            1233/411
ACT CGT GCA GCT GTT GAA GAA GGT ATT-GTT GCA GGT GGT GGA ACA GCT CTT GCC AAT GTG
T   R   A   A   V   E   E   G   I   V   A   G   G   G   T   A   L   A   N   V
```

FIG. 15A

```
1263/421                                                1293/431
ATT CCA GCT GTT GCT ACC TTG GAA TTG ACA GGA GAT GAA GCA ACA GGA CGT AAT ATT GTT
I   P   A   V   A   T   L   E   L   T   G   D   E   A   T   G   R   N   I   V
1323/441                                                1353/451
CTC CGT GCT TTG GAA GAA CCT GTT CGT CAA ATT GCT CAC AAT GCA GGA TTT GAA GGA TCT
L   R   A   L   E   E   P   V   R   Q   I   A   H   N   A   G   F   E   G   S
1383/461                                                1413/471
ATC GTT ATC GAT CGT TTG AAA AAT GCT GAG CTT GGT ATA GGA TTC AAC GCA GCA ACT GGC
I   V   I   D   R   L   K   N   A   E   L   G   I   G   F   N   A   A   T   G
1443/481                                                1473/491
GAG TGG GTT AAC ATG ATT GAT CAA GGT ATC ATT GAT CCA GTT AAA GTG AGT CGT TCA GCC
E   W   V   N   M   I   D   Q   G   I   I   D   P   V   K   V   S   R   S   A
1503/501                                                1533/511
CTA CAA AAT GCA GCA TCT GTA GCC AGC TTG ATT TTG ACA ACA GAA GCA GTC GTA GCC AAT
L   Q   N   A   A   S   V   A   S   L   I   L   T   T   E   A   V   V   A   N
1563/521                                                1593/531
AAA CCA GAA CCA GTA GCC CCA GCT CCA GCA ATG GAT CCA AGT ATG ATG GGT GGA ATG GGC
K   P   E   P   V   A   P   A   P   A   M   D   P   S   M   M   G   G   M   G
1623/541                                                1653/551
GGA GCT AGC atg cat gga gat aca cct aca ttg cat gaa tat atg tta gat ttg caa cca
G   A   S   M   H   G   D   T   P   T   L   H   E   Y   M   L   D   L   Q   P
1683/561                                                1713/571
gag aca act gat ctc tac tgt tat gag caa tta aat gac agc tca gag gag gag gat gaa
E   T   T   D   L   Y   C   Y   E   Q   L   N   D   S   S   E   E   E   D   E
1743/581                                                1773/591
ata gat ggt cca gct gga caa gca gaa ccg gac aga gcc cat tac aat att gta acc ttt
I   D   G   P   A   G   Q   A   E   P   D   R   A   H   Y   N   I   V   T   F
1803/601                                                1833/611
tgt tgc aag tgt gac tct acg ctt cgg ttg tgc gta caa agc aca cac gta gac att cgt
C   C   K   C   D   S   T   L   R   L   C   V   Q   S   T   H   V   D   I   R
1863/621                                                1893/631
act ttg gaa gac ctg tta atg ggc aca cta gga att gtg tgc ccc atc tgt tct cag aaa
T   L   E   D   L   L   M   G   T   L   G   I   V   C   P   I   C   S   Q   K
1923/641
cca TAA
P   *
```

FIG. 15B

```
4/1                                                                 34/11
ATG AAA GAG CTC AAG TTC GGT GTC GAA GCC CGT GCT CAG CTC CTC AAG GGT GTT GAC ACT
M   K   E   L   K   F   G   V   E   A   R   A   Q   L   L   K   G   V   D   T
64/21                                                               94/31
CTG GCC AAG GCC GTG ACT TCG ACT CTT GGT CCT AAG GGT CGT AAC GTC CTT ATC GAG TCT
L   A   K   A   V   T   S   T   L   G   P   K   G   R   N   V   L   I   E   S
124/41                                                              154/51
CCC TAT GGC TCC CCT AAG ATC ACC AAG GAT GGT GTC TCT GTT GCC AAG GCC ATC ACT CTC
P   Y   G   S   P   K   I   T   K   D   G   V   S   V   A   K   A   I   T   L
184/61                                                              214/71
CAA GAC AAG TTC GAG AAC CTC GGT GCT CGC CTC CTC CAG GAT GTC GCT TCT AAG ACC AAC
Q   D   K   F   E   N   L   G   A   R   L   L   Q   D   V   A   S   K   T   N
244/81                                                              274/91
GAG ATT GCT GGT GAC GGT ACC ACC ACC GCT ACC GTC CTT GCC CGT GCC ATC TTC TCT GAG
E   I   A   G   D   G   T   T   T   A   T   V   L   A   R   A   I   F   S   E
304/101                                                             334/111
ACC GTG AAG AAT GTT GCT GCT GGC TGC AAC CCC ATG GAT CTG CGC CGC GGT ATC CAG GCT
T   V   K   N   V   A   A   G   C   N   P   M   D   L   R   R   G   I   Q   A
364/121                                                             394/131
GCT GTT GAT GCT GTC GTC GAC TAC CTC CAG AAG AAC AAG CGT GAC ATC ACC ACC GGT GAG
A   V   D   A   V   V   D   Y   L   Q   K   N   K   R   D   I   T   T   G   E
424/141                                                             454/151
GAG ATC GCT CAG GTT GCT ACT ATC TCC GCT AAC GGT GAC ACC CAC ATT GGT AAG CTG ATC
E   I   A   Q   V   A   T   I   S   A   N   G   D   T   H   I   G   K   L   I
484/161                                                             514/171
TCC ACC GCC ATG GAG CGT GTT GGC AAG GAG GGT GTC ATC ACT GTC AAG GAG GGC AAG ACC
S   T   A   M   E   R   V   G   K   E   G   V   I   T   V   K   E   G   K   T
544/181                                                             574/191
ATT GAG GAT GAG CTC GAG GTC ACT GAG GGT ATG CGC TTC GAC CGT GGA TAC ACC TCC CCC
I   E   D   E   L   E   V   T   E   G   M   R   F   D   R   G   Y   T   S   P
604/201                                                             634/211
TAC TTC ATC ACC GAT ACC AAG TCC CAG AAG GTT GAG TTC GAG AAG CCT CTG ATT CTG CTG
Y   F   I   T   D   T   K   S   Q   K   V   E   F   E   K   P   L   I   L   L
664/221                                                             694/231
TCT GAG AAG AAG ATC TCT GCC GTT CAG GAC ATC ATC CCC GCC CTT GAG GCC TCC ACC ACC
S   E   K   K   I   S   A   V   Q   D   I   I   P   A   L   E   A   S   T   T
724/241                                                             754/251
CTC CGC CGC CCC CTG GTT ATT ATC GCA GAG GAC ATT GAG GGT GAG GCT CTC GCC GTC TGC
L   R   R   P   L   V   I   I   A   E   D   I   E   G   E   A   L   A   V   C
784/261                                                             814/271
ATT CTG AAC AAG CTT CGT GGC CAG CTG CAG GTC GCT GCT GTC AAG GCT CCT GGA TTC GGT
I   L   N   K   L   R   G   Q   L   Q   V   A   A   V   K   A   P   G   F   G
844/281                                                             874/291
GAC AAC CGC AAG AGC ATC CTG GGC GAT CTT GCC GTC CTT ACC AAC GGT ACC GTC TTC ACT
D   N   R   K   S   I   L   G   D   L   A   V   L   T   N   G   T   V   F   T
904/301                                                             934/311
GAT GAG CTC GAC ATC AAA CTC GAG AAG CTT ACC CCC GAT ATG CTT GGT TCC ACC GGC GCC
D   E   L   D   I   K   L   E   K   L   T   P   D   M   L   G   S   T   G   A
964/321                                                             994/331
ATC ACC ATC ACC AAG GAG GAC ACC ATC ATC CTG AAC GGG GAG GGC AGC AAG GAC GCC ATT
I   T   I   T   K   E   D   T   I   I   L   N   G   E   G   S   K   D   A   I
1024/341                                                            1054/351
GCC CAG CGC TGC GAG CAG ATT CGC GGT GTC ATG GCG GAC CCC AGC ACC TCC GAA TAC GAG
A   Q   R   C   E   Q   I   R   G   V   M   A   D   P   S   T   S   E   Y   E
1084/361                                                            1114/371
AAG GAG AAG CTC CAG GAG CGT CTA GCT AAG CTC TCT GGC GGT GTT GCC GTC ATC AAG GTC
K   E   K   L   Q   E   R   L   A   K   L   S   G   G   V   A   V   I   K   V
1144/381                                                            1174/391
GGT GGT GCC TCC GAG GTT GAG GTC GGT GAG AAG AAG GAC CGT GTT GTC GAT GCT CTC AAT
G   G   A   S   E   V   E   V   G   E   K   K   D   R   V   V   D   A   L   N
1204/401                                                            1234/411
GCT ACC CGT GCT GCT GTT GAG GAG GGT ATC CTC CCC GGT GGT GGT ACC GCC CTT CTC AAG
A   T   R   A   A   V   E   E   G   I   L   P   G   G   G   T   A   L   L   K
```

FIG. 16A

```
1264/421                                                1294/431
GCC GCC GCC AAC GGC CTT GAC AAT GTC AAG CCC GAG AAC TTC GAC CAG CAA CTC GGT GTG
A   A   A   N   G   L   D   N   V   K   P   E   N   F   D   Q   Q   L   G   V
1324/441                                                1354/451
AGC ATC ATC AAG AAT GCC ATC ACC CGC CCC GCT CGC ACC ATT GTT GAG AAC GCC GGC CTC
S   I   I   K   N   A   I   T   R   P   A   R   T   I   V   E   N   A   G   L
1384/461                                                1414/471
GAG GGC AGC GTC ATT GTC GGC AAG CTG ACC GAC GAG TTC GCC AAG GAC TTC AAC CGC GGT
E   G   S   V   I   V   G   K   L   T   D   E   F   A   K   D   F   N   R   G
1444/481                                                1474/491
TTC GAC AGC TCC AAG GGC GAG TAC GTC GAC ATG ATC TCC AGC GGT ATC CTC GAT CCC CTC
F   D   S   S   K   G   E   Y   V   D   M   I   S   S   G   I   L   D   P   L
1504/501                                                1534/511
AAG GTT GTT CGC ACC GCT CTG CTC GAC GCC AGC GGT GTC GCC TCC CTG CTC GGT ACC ACT
K   V   V   R   T   A   L   L   D   A   S   G   V   A   S   L   L   G   T   T
1564/521                                                1594/531
GAG GTC GCT ATT GTT GAG GCC CCT GAG GAG AAG GGC CCC GCT GCT CCT GGC ATG GGT GGT
E   V   A   I   V   E   A   P   E   E   K   G   P   A   A   P   G   M   G   G
1624/541                                                1654/551
ATG GGT GGT ATG GGC GGC ATG GGC GGC ATG CAT GGA GAT ACA CCT ACA TTG CAT GAA TAT
M   G   G   M   G   G   M   G   G   M   H   G   D   T   P   T   L   H   E   Y
1684/561                                                1714/571
ATG TTA GAT TTG CAA CCA GAG ACA ACT GAT CTC TAC TGT TAT GAG CAA TTA AAT GAC AGC
M   L   D   L   Q   P   E   T   T   D   L   Y   C   Y   E   Q   L   N   D   S
1744/581                                                1774/591
TCA GAG GAG GAG GAT GAA ATA GAT GGT CCA GCT GGA CAA GCA GAA CCG GAC AGA GCC CAT
S   E   E   E   D   E   I   D   G   P   A   G   Q   A   E   P   D   R   A   H
1804/601                                                1834/611
TAC AAT ATT GTA ACC TTT TGT TGC AAG TGT GAC TCT ACG CTT CGG TTG TGC GTA CAA AGC
Y   N   I   V   T   F   C   C   K   C   D   S   T   L   R   L   C   V   Q   S
1864/621                                                1894/631
ACA CAC GTA GAC ATT CGT ACT TTG GAA GAC CTG TTA ATG GGC ACA CTA GGA ATT GTG TGC
T   H   V   D   I   R   T   L   E   D   L   L   M   G   T   L   G   I   V   C
1924/641
CCC ATC TGT TCT CAG AAA CCA TAG
P   I   C   S   Q   K   P   *
```

FIG. 16B

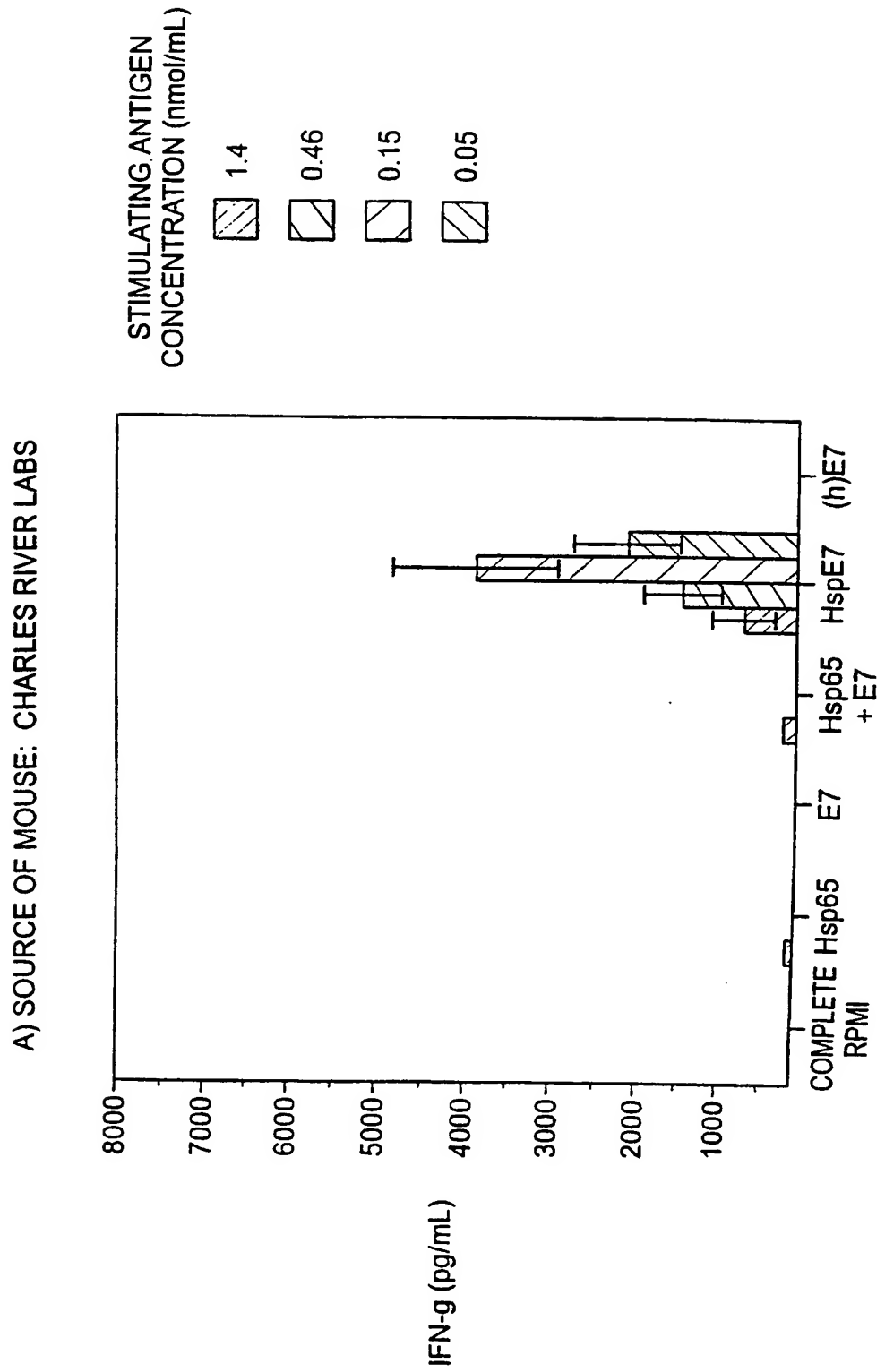
A) SOURCE OF MOUSE: CHARLES RIVER LABS
STIMULATING ANTIGEN CONCENTRATION (nmol/mL)
1.4
0.46
0.15
0.05
IFN-g (pg/mL)
8000
7000
6000
5000
4000
3000
2000
1000
COMPLETE RPMI
Hsp65
E7
Hsp65 + E7
HspE7
(h)E7
STIMULATING ANTIGEN

FIG. 17A

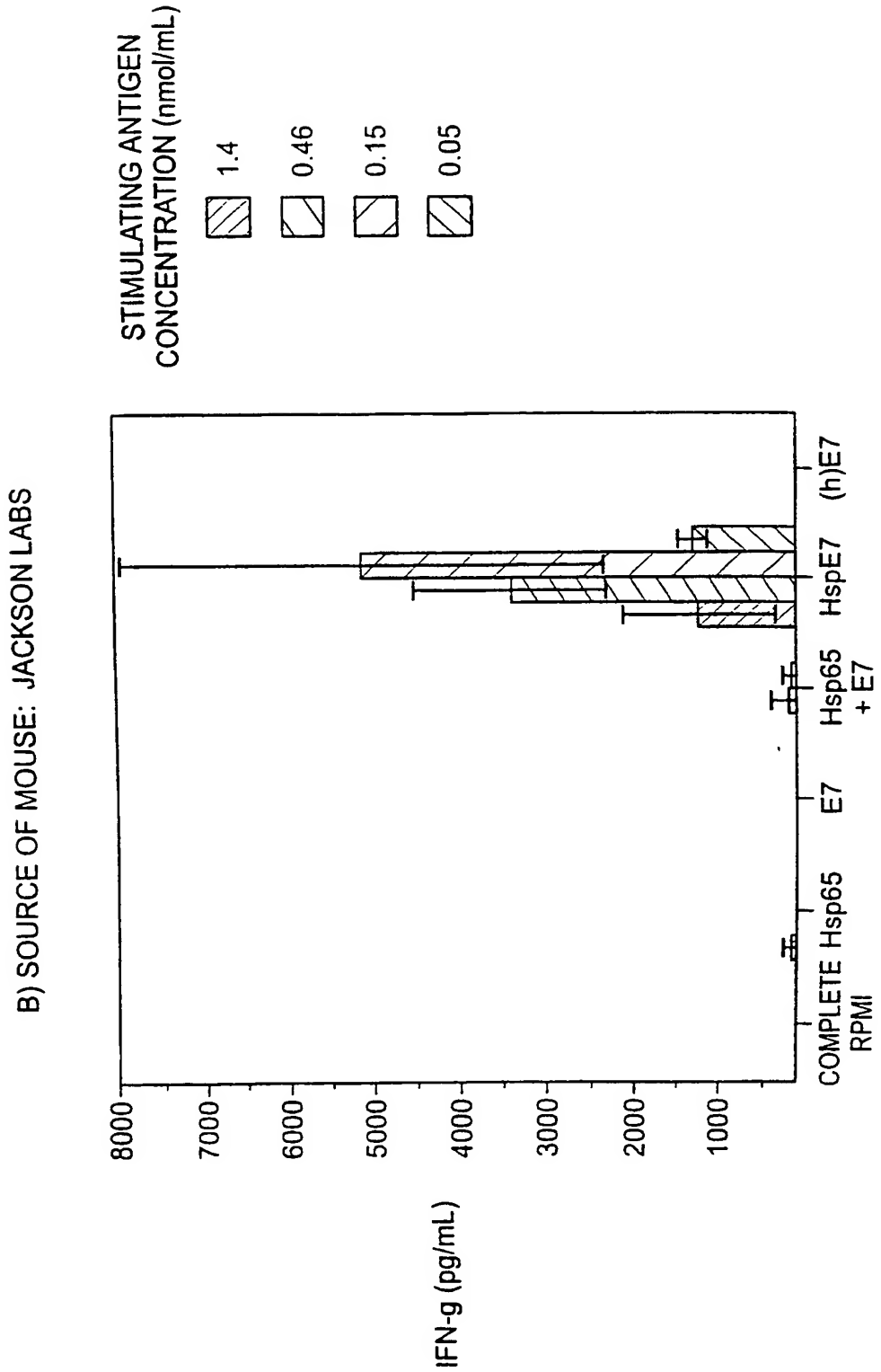
B) SOURCE OF MOUSE: JACKSON LABS
STIMULATING ANTIGEN CONCENTRATION (nmol/mL)
1.4
0.46
0.15
0.05
IFN-g (pg/mL)
8000
7000
6000
5000
4000
3000
2000
1000
COMPLETE RPMI
Hsp65
E7
Hsp65 + E7
HspE7
(h)E7
STIMULATING ANTIGEN

FIG. 17B

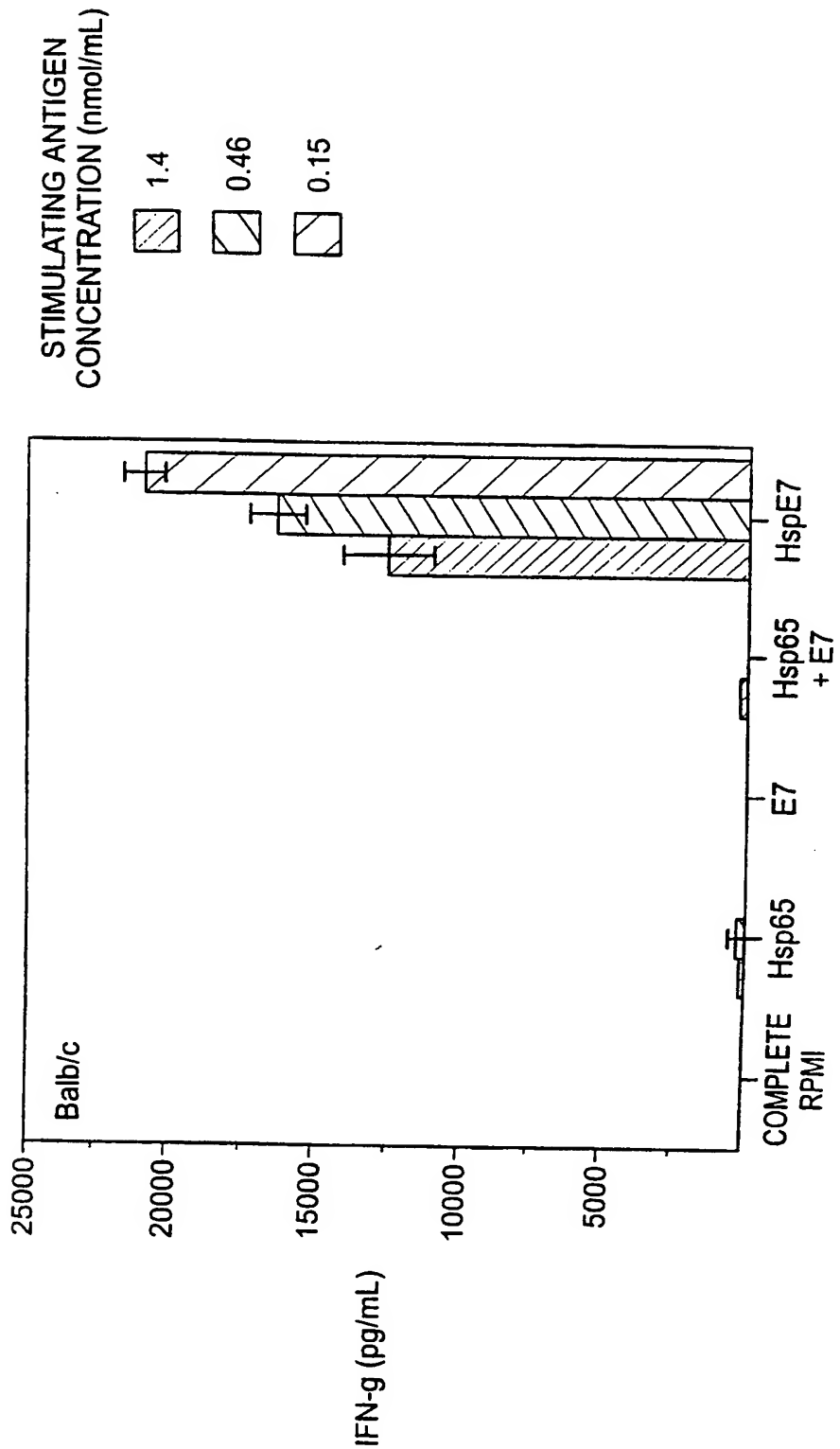
STIMULATING ANTIGEN CONCENTRATION (nmol/mL)
1.4
0.46
0.15
Balb/c
IFN-g (pg/mL)
25000
20000
15000
10000
5000
COMPLETE RPMI
Hsp65
E7
Hsp65 + E7
HspE7
STIMULATING ANTIGEN

FIG. 18A

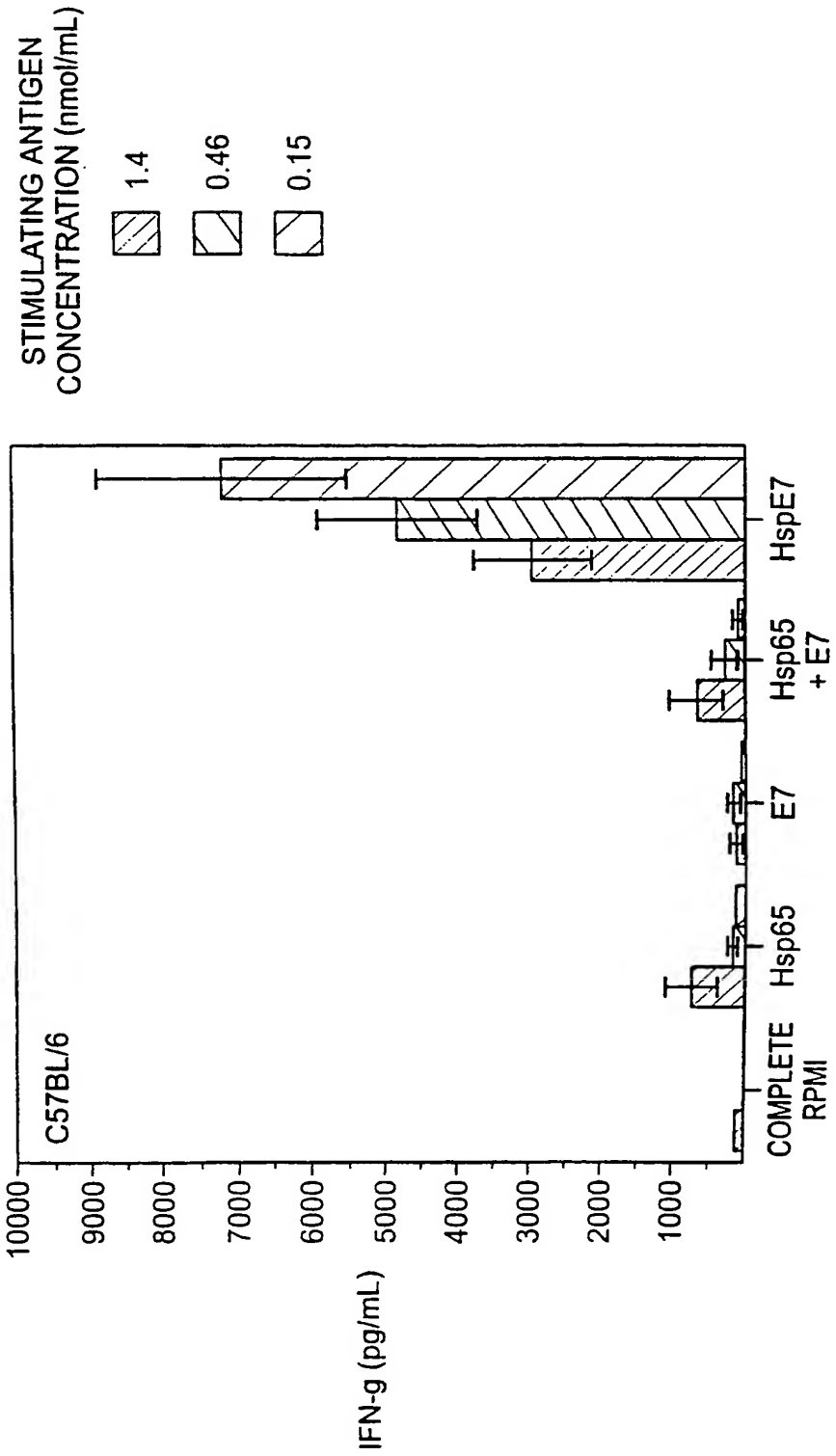
STIMULATING ANTIGEN CONCENTRATION (nmol/mL)
1.4
0.46
0.15
C57BL/6
IFN-g (pg/mL)
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
COMPLETE RPMI
Hsp65
E7
Hsp65 + E7
HspE7
STIMULATING ANTIGEN

FIG. 18B

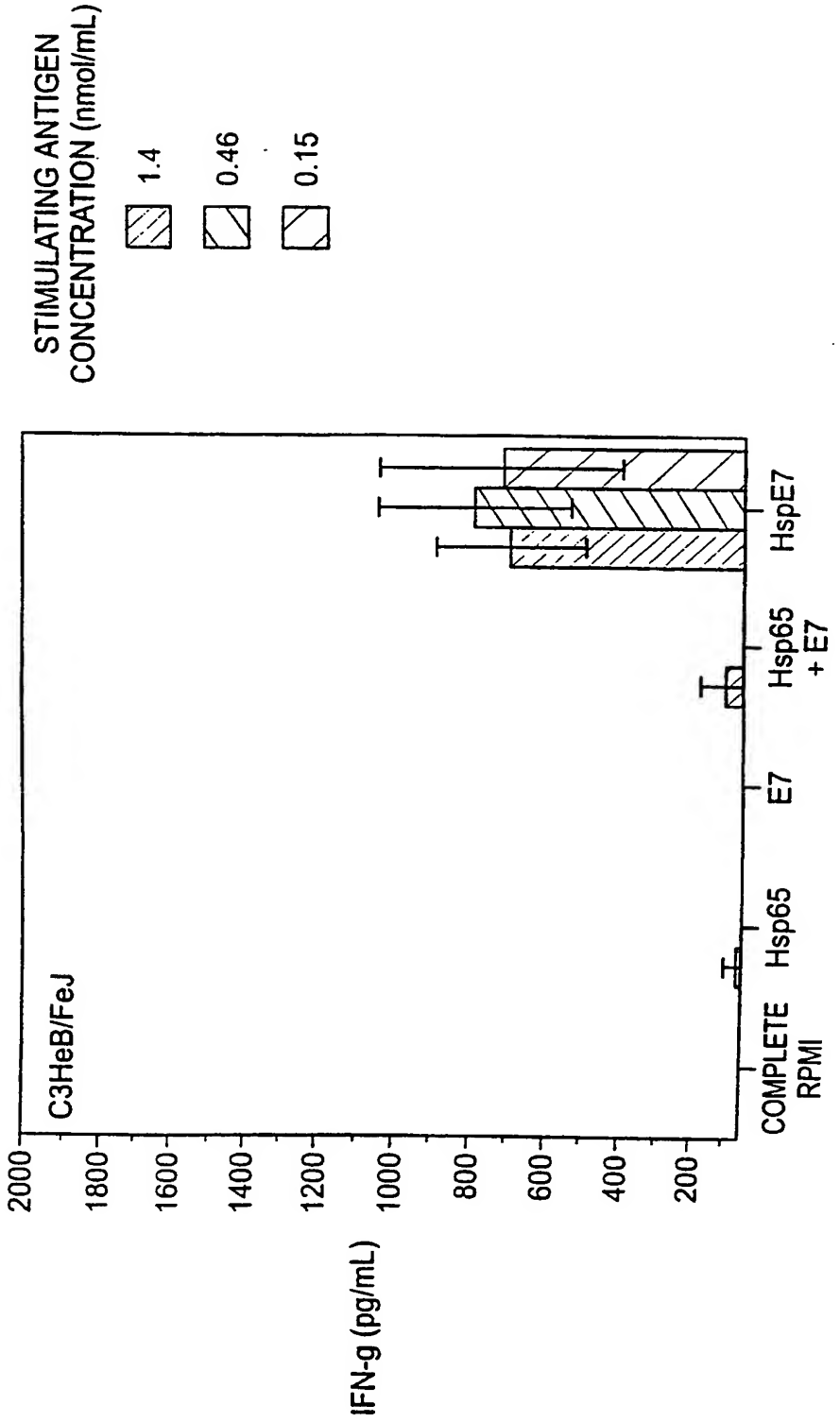
STIMULATING ANTIGEN CONCENTRATION (nmol/mL)
1.4
0.46
0.15
C3HeB/FeJ
IFN-g (pg/mL)
2000
1800
1600
1400
1200
1000
800
600
400
200
COMPLETE RPMI
Hsp65
E7
Hsp65 + E7
HspE7
STIMULATING ANTIGEN

FIG. 18C

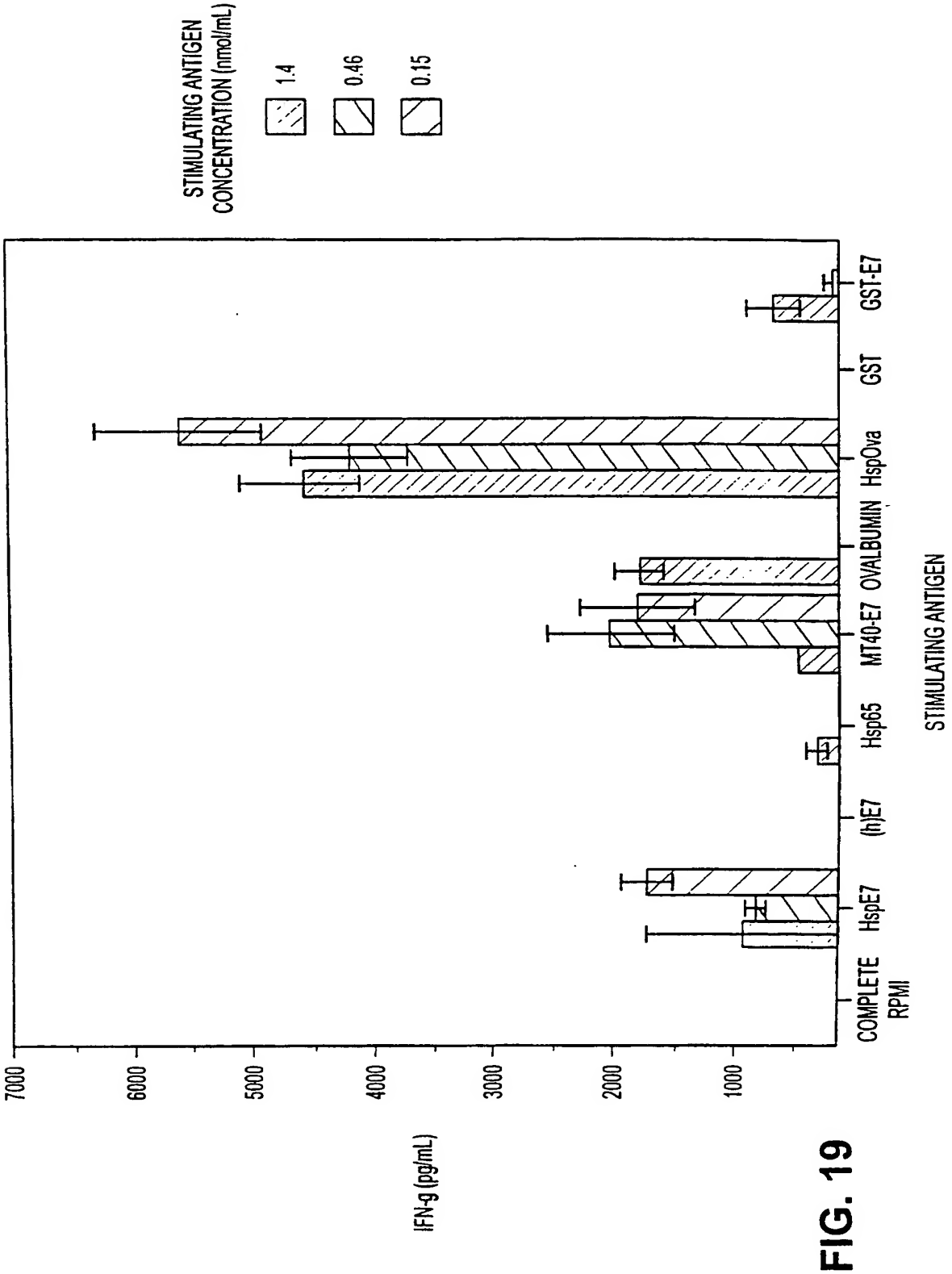
STIMULATING ANTIGEN CONCENTRATION (nmol/mL)
1.4
0.46
0.15
IFN-g (pg/mL)
7000
6000
5000
4000
3000
2000
1000
COMPLETE RPMI
HspE7
(h)E7
Hsp65
MT40-E7
OVALBUMIN
HspOva
GST
GST-E7
STIMULATING ANTIGEN

FIG. 19

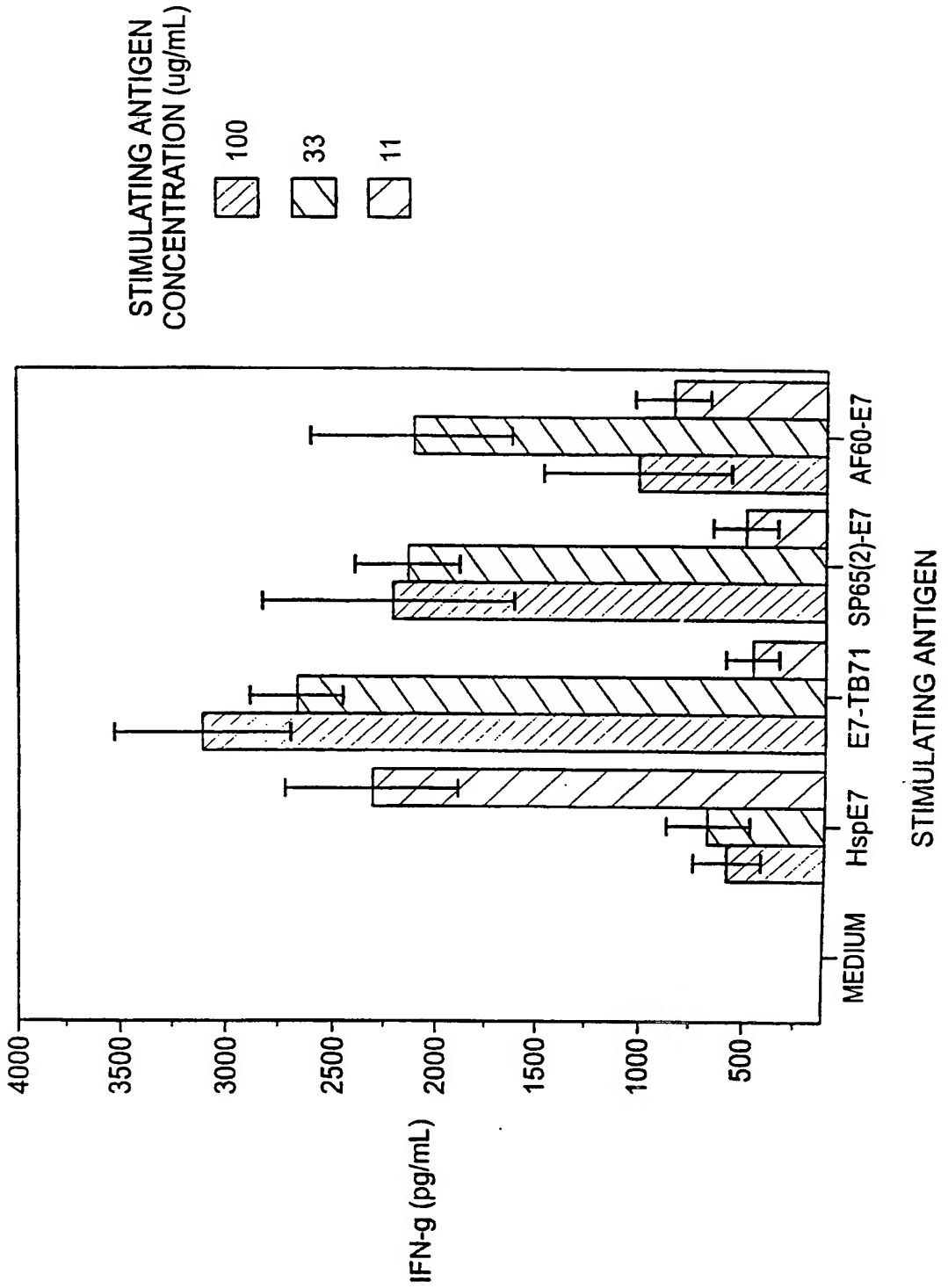
STIMULATING ANTIGEN CONCENTRATION (ug/mL)
100
33
11
IFN-g (pg/mL)
4000
3500
3000
2500
2000
1500
1000
500
MEDIUM
HspE7
E7-TB71
SP65(2)-E7
AF60-E7
STIMULATING ANTIGEN

FIG. 20A

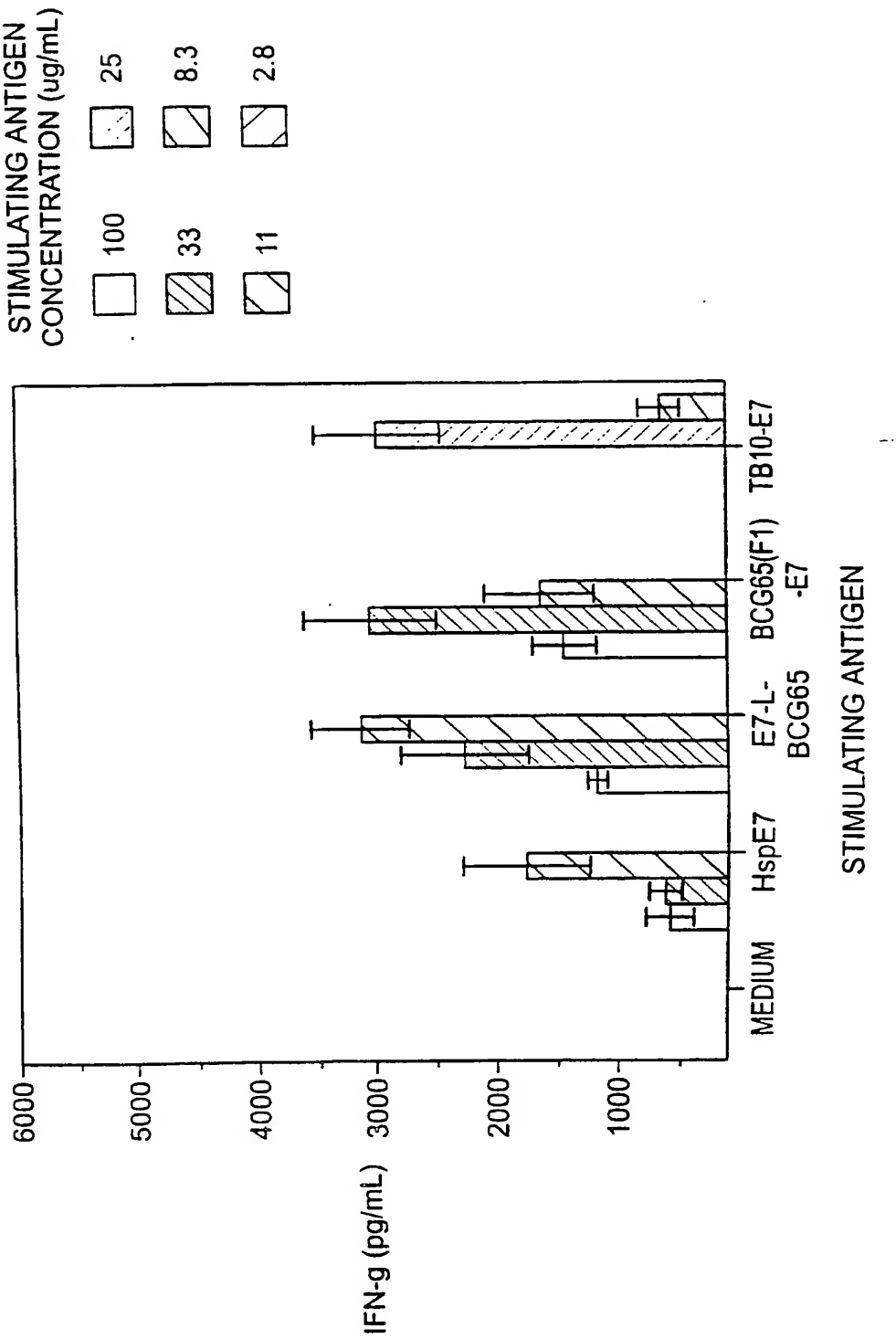
STIMULATING ANTIGEN CONCENTRATION (ug/mL)
100
33
11
25
8.3
2.8
IFN-g (pg/mL)
6000
5000
4000
3000
2000
1000
MEDIUM
HspE7
E7-L-BCG65
BCG65(F1)-E7
TB10-E7
STIMULATING ANTIGEN

FIG. 20B

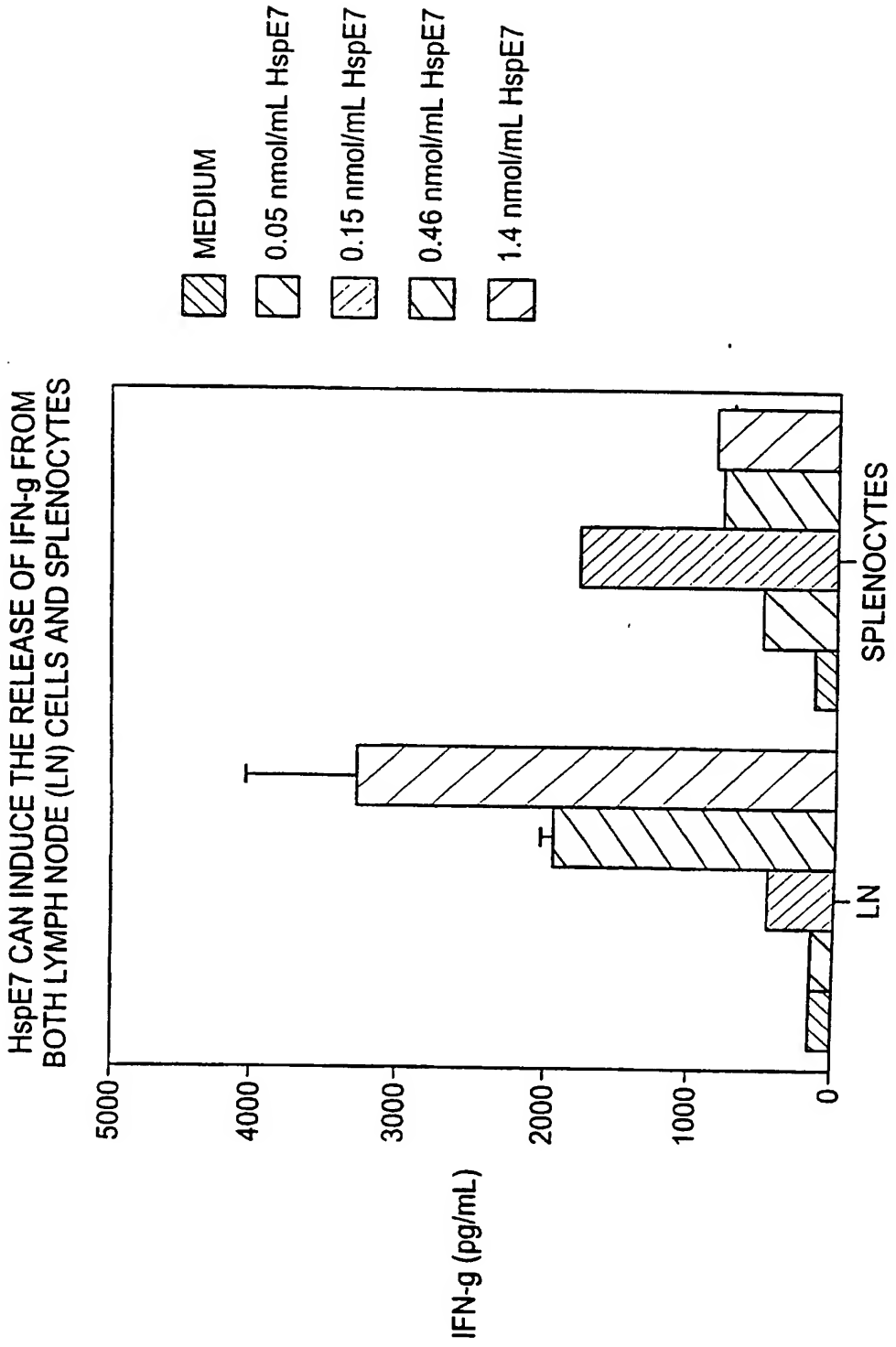

FIG. 21

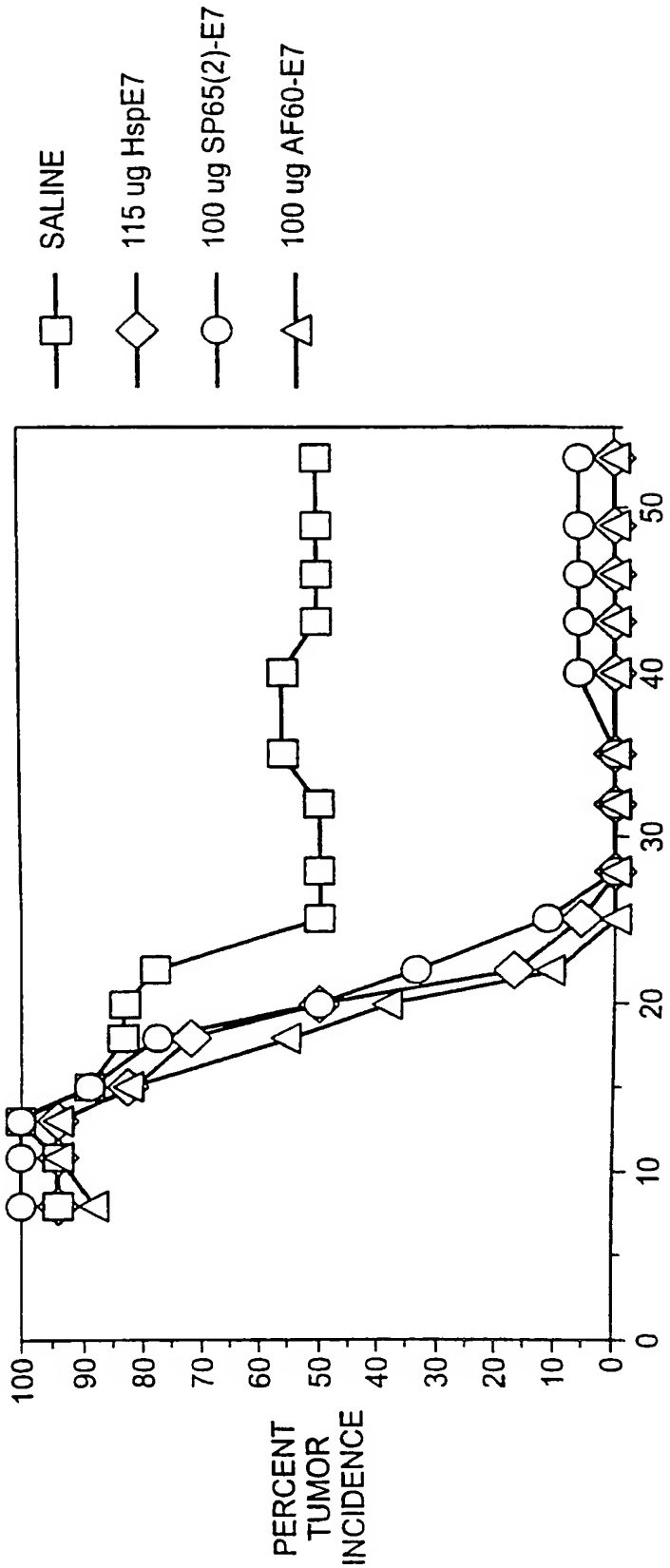
SALINE
115 ug HspE7
100 ug SP65(2)-E7
100 ug AF60-E7
PERCENT TUMOR INCIDENCE
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
DAY AFTER INJECTION OF TC-1 TUMOR CELLS

FIG. 22A

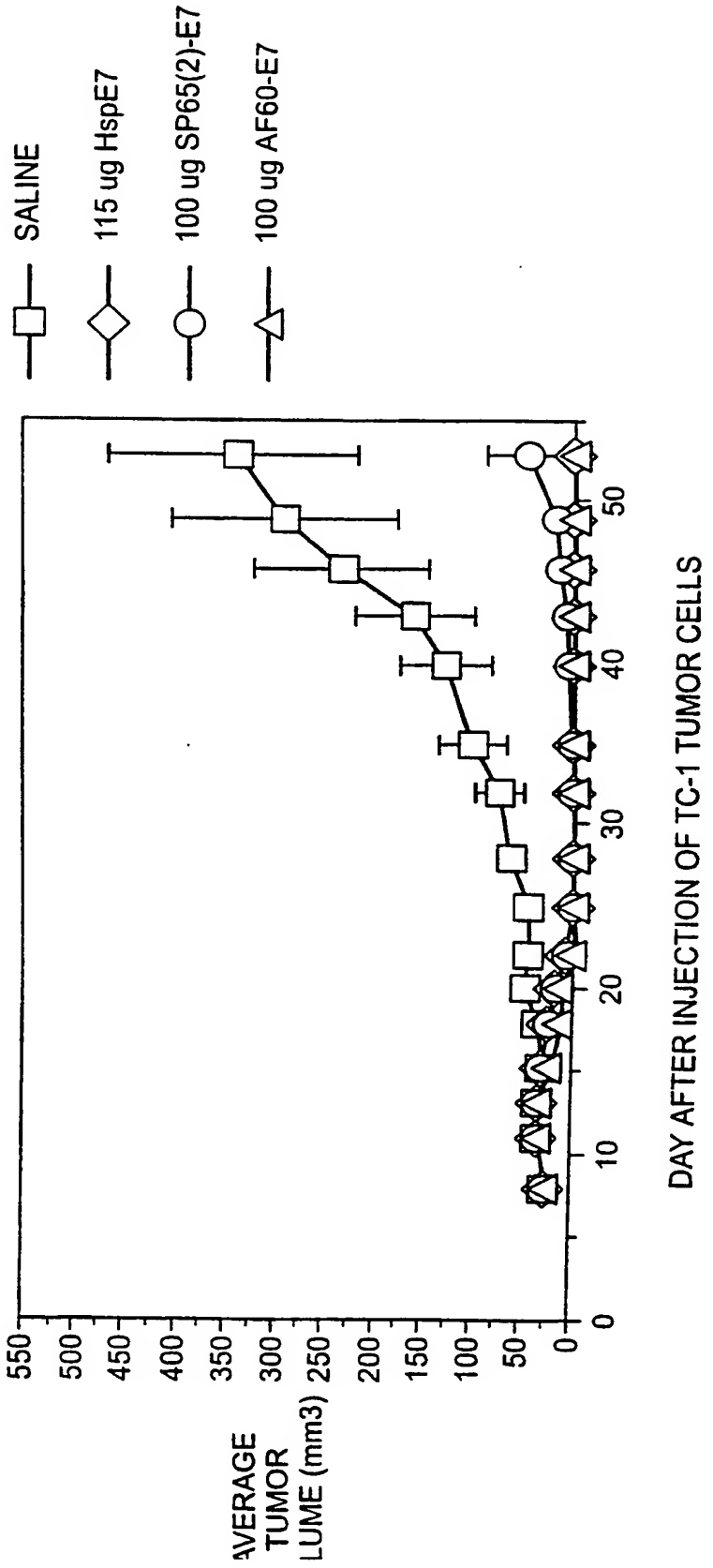
SALINE
115 ug HspE7
100 ug SP65(2)-E7
100 ug AF60-E7
AVERAGE TUMOR LUME (mm3)
550
500
450
400
350
300
250
200
150
100
50
0
0
10
20
30
40
50
DAY AFTER INJECTION OF TC-1 TUMOR CELLS

FIG. 22B

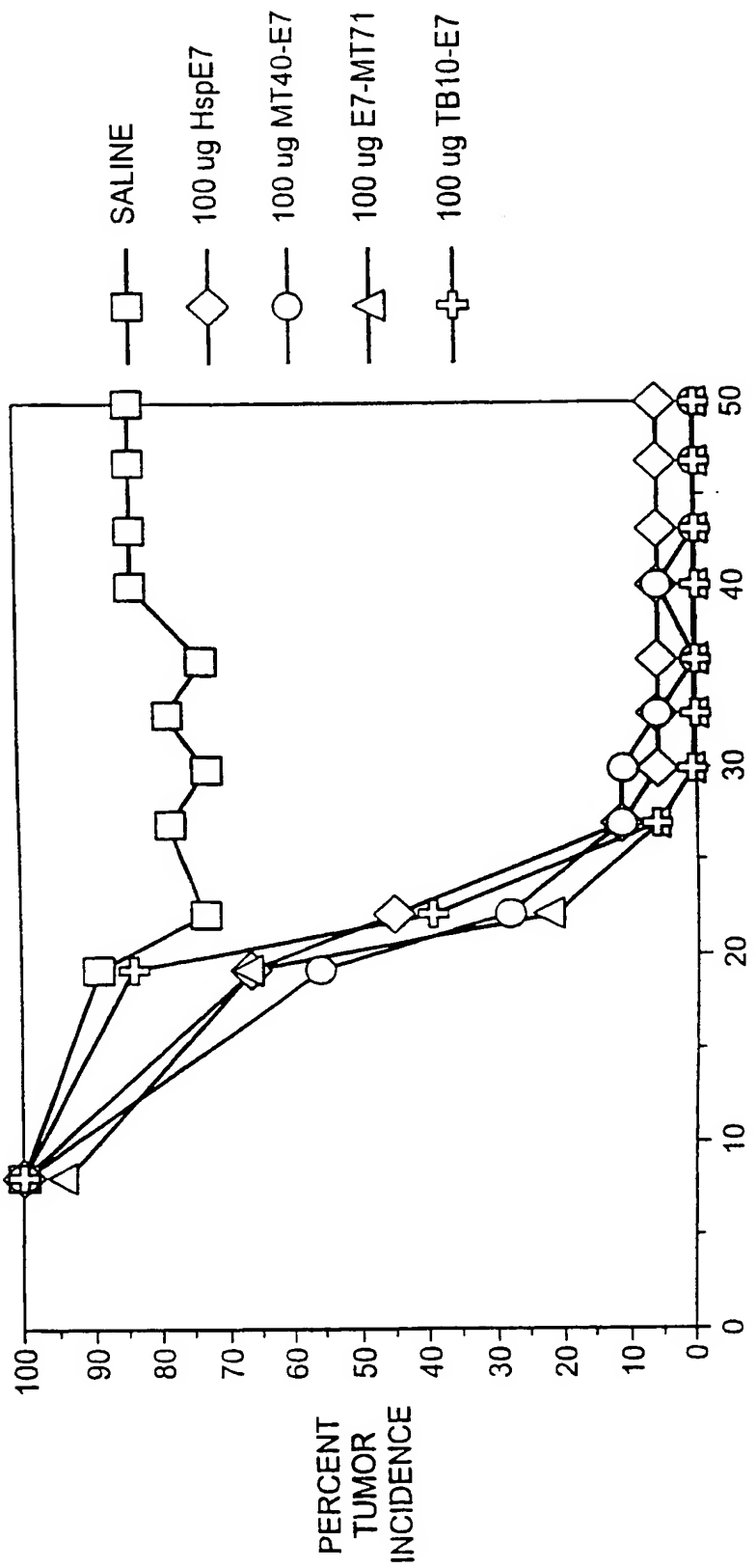
SALINE
100 ug HspE7
100 ug MT40-E7
100 ug E7-MT71
100 ug TB10-E7
PERCENT TUMOR INCIDENCE
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
DAYS AFTER INJECTION OF TC-1 TUMOR CELLS

FIG. 23A

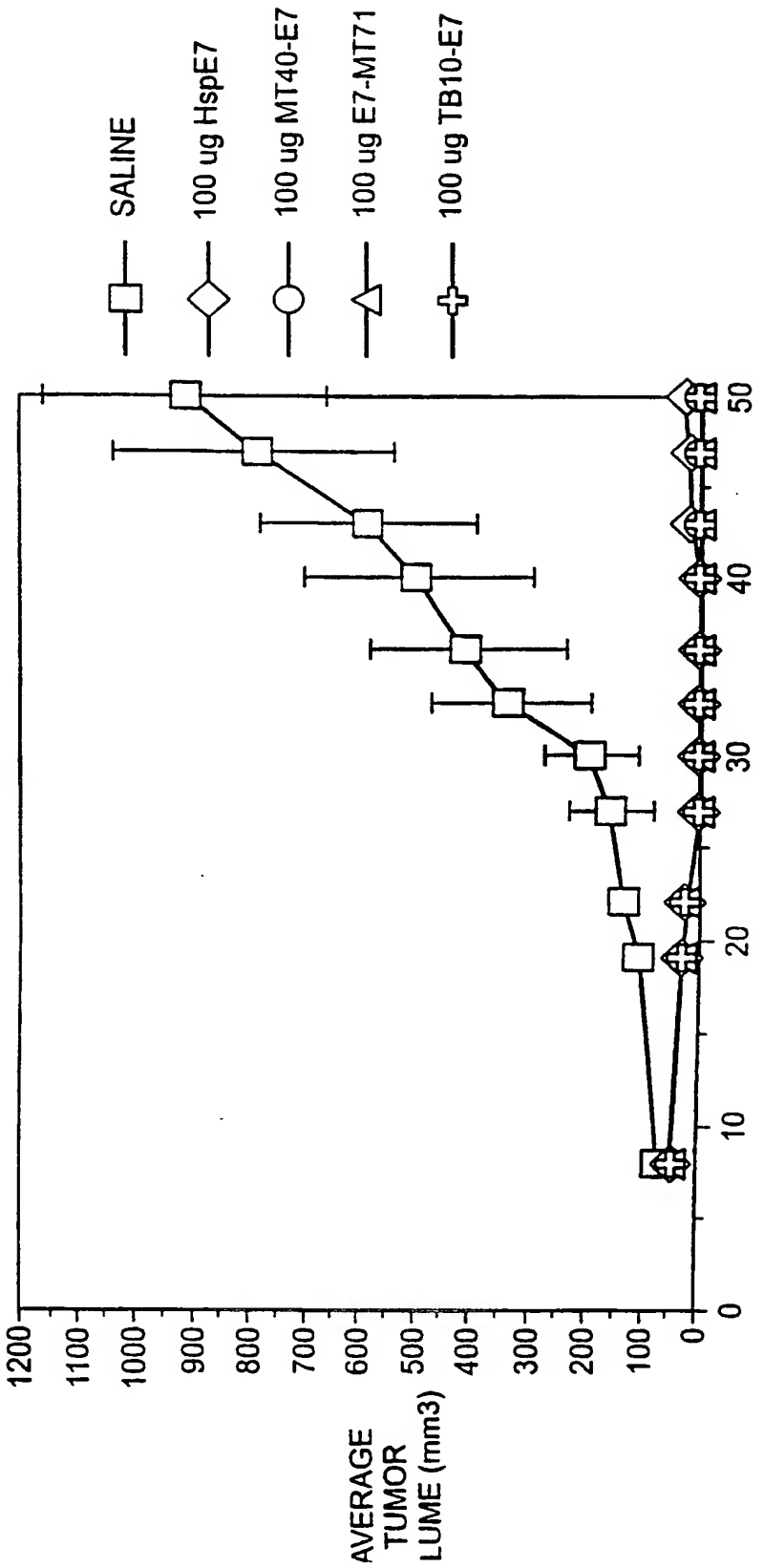
SALINE
100 ug HspE7
100 ug MT40-E7
100 ug E7-MT71
100 ug TB10-E7
AVERAGE TUMOR LUME (mm3)
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
0
10
20
30
40
50
DAYS AFTER INJECTION OF TC-1 TUMOR CELLS

FIG. 23B

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(71) Applicant *(for all designated States except US)*: **STRESSGEN BIOTECHNOLOGIES CORPORATION** [CA/CA]; 350-4243 Glanford Avenue, Victoria, British Columbia V8Z 4B9 (CA).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **SIEGEL, Marvin** [US/US]; 150 Somerset Drive, Blue Bell, PA 19422 (US). **CHU, N., Randall** [CA/CA]; 2225 Windsor Road, Victoria, British Columbia V8S 3C8 (CA). **MIZZEN, Lee, A.** [CA/CA]; 1936 Quamichan Street, Victoria, British Columbia V8S 2C4 (CA).

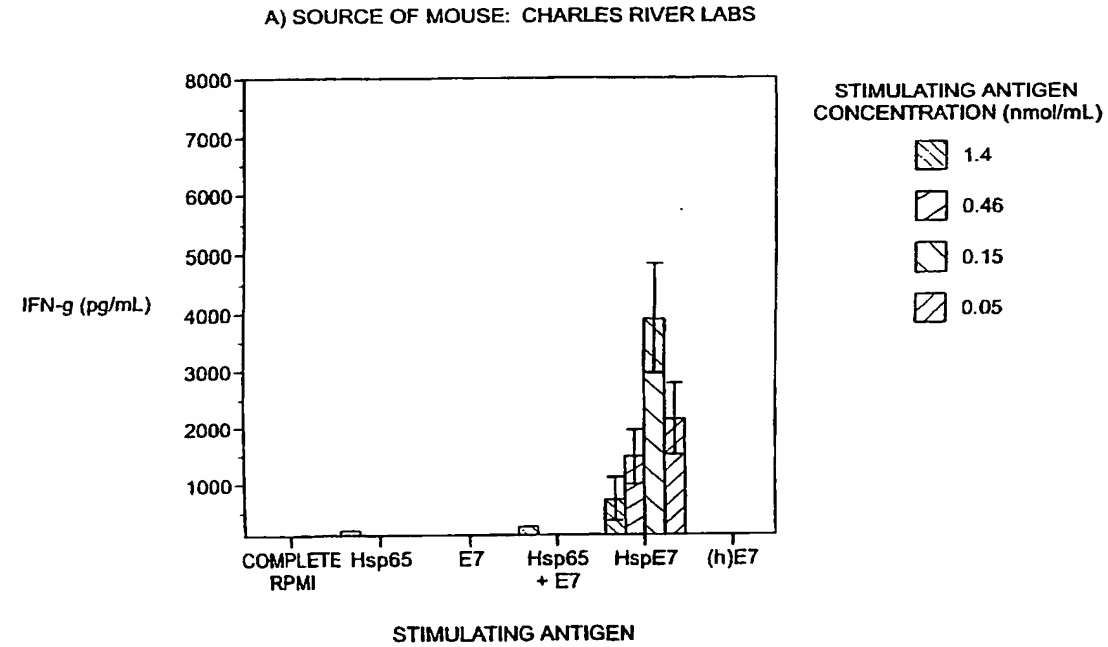
(74) Agent: **FRASER, Janis, K.**; Fish & Richardson, P.C., 225 Franklin Street, Boston, MA 02110-2804 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,

[Continued on next page]

(54) Title: INDUCTION OF A TH1-LIKE RESPONSE IN VITRO



(57) Abstract: The invention provides compositions and methods for stimulating a Th1-like response *in vitro*. Compositions include fusion proteins and conjugates that contain at least a portion of a heat shock protein. A Th1-like response can be elicited by contacting *in vitro* a cell sample containing naive lymphocytes with a fusion protein or conjugate of the invention. The Th1-like response can be detected by measuring IFN-gamma produced by the cell sample.

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IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 00/18828

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/50 C07K14/35 C07K14/025 C12N15/62

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, MEDLINE, EPO-Internal, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 December 2000	12. 06. 2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Gundlach, B

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INTERNATIONAL SEARCH REPORT

Inte. ɔnal Application No
PCT/US 00/18828

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Int ional Application No
PCT/US 00/18828

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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T	CHU N R ET AL: "Immunotherapy of a human papillomavirus (HPV) type 16 E7-expressing tumour by administration of fusion protein comprising Mycobacterium bovis bacille Calmette-Guerin (BCG) hsp65 and HPV16 E7." CLINICAL AND EXPERIMENTAL IMMUNOLOGY, vol. 121, no. 2, August 2000 (2000-08), pages 216-225, XP000965423 ISSN: 0009-9104 the whole document -----	1-34, 39-42,64

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